PHYLOGEOGRAPHY, ECOGEOGRAPHIC VARIATION, AND EVOLUTIONARY HISTORY OF THE COLLARED PIKA (*OCHOTONA COLLARIS*)

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PHYLOGEOGRAPHY, ECOGEOGRAPHIC VARIATION, AND EVOLUTIONARY HISTORY OF THE COLLARED PIKA (*OCHOTONA COLLARIS*)

A

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By

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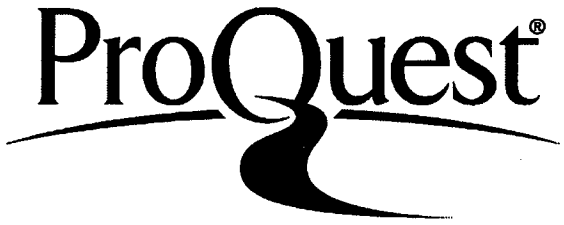

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Abstract

In this dissertation I address the evolutionary history, ecogeographic variation, and phylogeography (single species and comparative) of collared pikas (*Ochotona collaris*). Pikas are small (ca. 150 g) lagomorphs (order Lagomorpha: rabbits, hares, and pikas) found in alpine habitats throughout much of the Holarctic. Only two of the 30 extant pika species occur in the New World. The northern of the two species, *O. collaris* is separated 800 km north of the American pika (*O. princeps*). In the first chapter, I employ recently developed molecular analytical techniques to examine when the two North American species diverged. This chapter sets the evolutionary context for the subsequent diversification within *O. collaris*. In the second chapter, I take a finer-scale view of morphological and ecogeographic variation in the collared pika. I examined morphological variation along a latitudinal gradient and over the past half century. While the length of the growing season appears to best explain latitudinal trends, temporal changes in body size are better explained by interrelated pressures resulting from heat stress and loss of snow cover. In the third chapter, I assess the major phylogeographic patterns within *O. collaris* and compare the observed levels of diversity within *O. collaris* to those in other alpine-adapted small mammal species. There are two main phylogroups in *O. collaris*, which appear to be in contact in Wrangell-St. Elias National Park and Preserve. The within-phylogroup and within-species genetic diversity in *O. collaris* is lower than that observed in other pikas. In the fourth chapter, I contrast the two *O. collaris* phylogroups with similar patterns in four co-distributed arctic-alpine small mammals in a comparative phylogeographic context. Although there are differences in the amount of diversity and extent of each phylogroup, simultaneous divergence into similarly distributed intraspecific phylogroups in all five species is supported. Four of the species, including *O. collaris*, show evidence of Pleistocene population expansion followed by recent demographic decline. Throughout the

dissertation, I focus on the role of location (latitude) and time frame in geographically structuring genetic and morphological variation.

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General Introduction:

The geographic distribution of variation within a species is affected by abiotic and biotic phenomena that influence microevolutionary processes (e.g., migration, mutation, selection, genetic drift), over historical and contemporary timescales. The distribution of variation can be informative about the evolutionary origins, demographic history and trajectory, and selective pressures occurring within a species (Avise, 2009). Because organismal responses to climate change can be idiosyncratic (Hadly *et al.*, 2004), the comparison of patterns between closely related and/or codistributed species can provide important perspective (Hickerson *et al.*, 2010). Contextualizing within-species diversity can allow biogeographers to separate idiosyncratic patterns from commonalities between species. These commonalities can lead to insights regarding regions of endemism, range expansion, and past responses to climate change (Avise, 2000).

Collared pikas (*Ochotona collaris*) are small (ca. 150 g) members of the mammalian order Lagomorpha, which includes rabbits, hares, and pikas. The order Lagomorpha is sister to the most speciose mammalian order, Rodentia (Murphy *et al.*, 2001). Whereas Rodentia comprises more than 2,000 extant species, Lagomorpha is represented by fewer than 80 worldwide, of which 30 are pikas (Family Ochotonidae; Hoffmann & Smith, 2005; Wilson & Reeder, 2005). The majority of ecological, behavioral, and species diversity in pikas is found in Asia, with only two extant species occurring in North America (Smith, 2008). The collared pika and the American pika (*O. princeps*) are both talus-dwelling and restricted to mountainous regions in the western regions of North America (MacDonald & Jones, 1987; Smith & Weston, 1990). Based on the number of described subspecies within each species (36 in *O. princeps*, none in *O. collaris*; Hall, 1981), collared pikas might be considered to be the least variable member of a North American clade within a species-poor family and order.

Collared pikas are distributed on the talus slopes of Alaska and northwest Canada (MacDonald & Jones, 1987). They are diurnal herbivores and can be readily seen collecting and caching vegetation throughout the summer (Morrison, 2006). Each individual maintains a territory and a vegetation cache ("haypile") and will fiercely defend their resources from kleptoparasitism (raids by conspecifics; Smith *et al.*, 1990; McKechnie *et al.*, 1994). Pikas remain active during the winter months and rely on their haypiles for food and the snowpack for insulation (Morrison *et al.*, 2009; Beever *et al.*, 2010). Both North American pika species are generally considered to be highly philopatric (Peacock & Ray, 2001), and assessments of within-site movements of collared pikas support this trend (Franken & Hik, 2004). This has implications for the degree of population connectivity (Peacock, 1997).

Present connectivity between pika populations is also likely to have been strongly affected by Quaternary climate cycling (Galbreath *et al.*, 2010). Populations that are currently allopatric may have originated from the same ancestral source population during glacial cycles (Knowles, 2000). Many of the regions where collared pikas are currently found were repeatedly glaciated during the Pleistocene (Manley & Kaufman, 2002). Post-glacial recolonization models suggest that previously glaciated regions will show depressed genetic diversity relative to unglaciated regions with a long history of occupation (Hewitt, 2004). The genetic diversity in a population is strongly affected by the length of time a population has been present in a region and the long-term effective population size (Allendorf & Luikart, 2007).

To examine the temporal and geographic factors structuring diversity within collared pikas, I employed a series of genetic, morphological, and analytical techniques on preexisting museum specimens and on specimens collected for my dissertation. Field sampling was intended to target locations from which collared pikas were suspected but undocumented or areas with few to no available frozen tissues amenable to genetic analysis. Although absence of evidence is not evidence of absence, collecting trips to the Kenai Peninsula, the Nulato Hills, Elephant

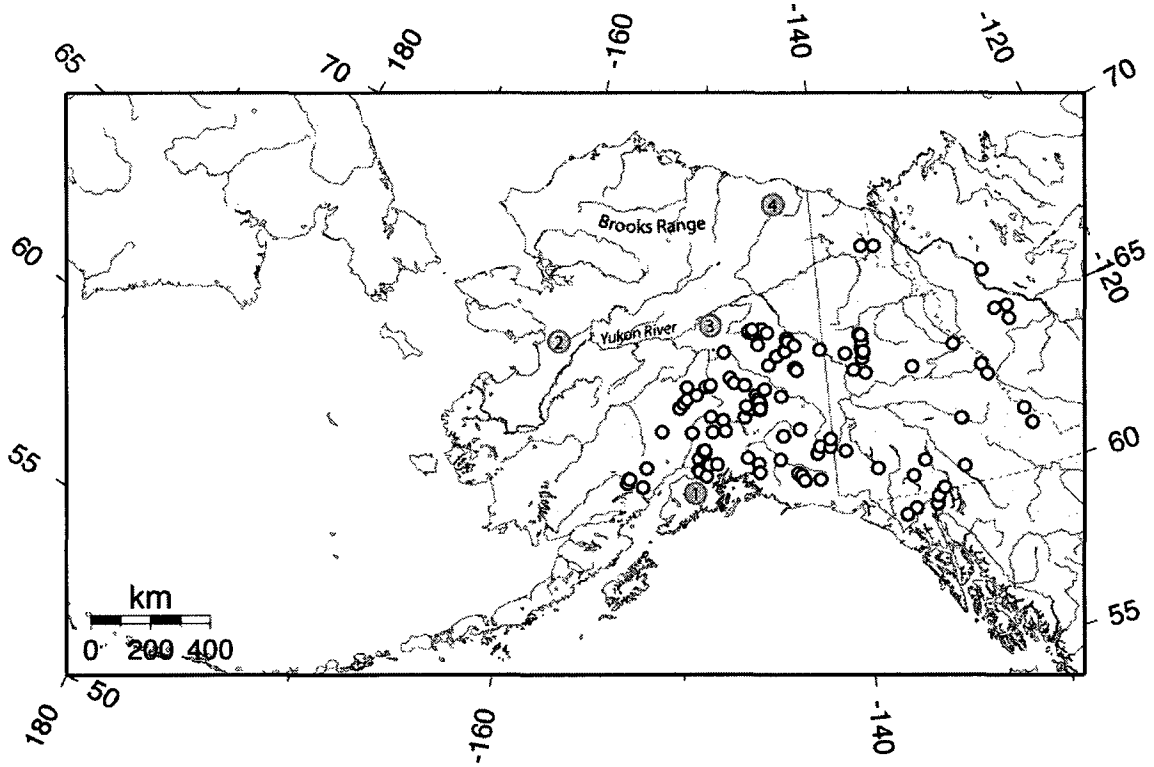

Figure 1 – Localities of vouchered museum specimens of collared pikas in major North American museums (data from MaNIS [http://manisnet.org/] and Chapters 2 & 3 of this study). Localities from which collared pikas were not found are also shown: 1) Kenai Peninsula, 2) Nulato Hills, 3) Elephant Mountain, and 4) the eastern Brooks Range.

Mountain (south of the Yukon River), and the Brooks Range failed to uncover any evidence of current or recent pika activity (Localities 1-4, Figure 1). Anecdotal reports of collared pikas in the eastern Brooks Range and the Kuskokwim Mountains have been noted for decades (Dufresne, 1955; MacDonald & Jones, 1987; MacDonald & Cook, 2009). Field collecting was successful in the White Mountains, at several localities in the Alaska Range, in the Chugach Mountains near Anchorage, and in Wrangell-St. Elias National Park and Preserve. Within Alaska, there are no substantiated records of *O. collaris* north of the Yukon River (Figure 1). However, they occur north of the Yukon River in the Ogilvie Mountains of the neighboring Yukon Territory and may be present in contiguous upland regions in Yukon-Charley

Rivers National Preserve. The eastern distributional limits of *O. collaris* have yet to be well defined, but the furthest east records occur in the MacKenzie Mountains of the Northwest Territories. Southernmost records of *O. collaris* occur in the Coast Mountains in the northwest tip of British Columbia (Swarth, 1936).

Recent climate change also plays a role in structuring genetic and morphological variation within many species (Hampe & Petit, 2005; Yom-Tov & Yom-Tov, 2005). Genetic and demographic signatures of population decline at lower latitude range margins are likely (Beever *et al.*, 2010). Many species also show evidence of body size changes that have been attributed to climate change (Smith & Betancourt, 2003; Teplitsky *et al.*, 2008). My fieldwork was intended to sample range margins to examine evidence of population growth or decline. I also revisited (and resampled) one of the most well represented historic collecting sites for collared pikas. Locality information for most historically collected specimens of collared pikas is seldom sufficiently detailed to permit resurvey work or sufficiently large to provide a representative sample. This site along the Denali Highway was well sampled *and* well documented by Robert S. Spooner in 1962. Natural history museums archive biological diversity from a particular location at a particular point in time. While the geographic extent of a sample can be replicated, the temporal aspect provided by museum samples makes each specimen irreplaceable.

In my doctoral research, I conducted phylogenetic, ecogeographic, and phylogeographic analyses of collared pikas to understand the origin and distribution of variation within the species. The analyses relied heavily on museum samples and emphasized the importance of museums in archiving species and diversity from a particular point in time. The specific objectives of this research were to:

1) Determine the evolutionary context and timing of the diversification within *O. collaris*,
2) Examine morphological variation along latitudinal and temporal gradients and investigate the underlying causes of these trends

3) Assess phylogeographic structure and genetic diversity patterns within *O. collaris*
4) Compare the phylogeographic structure of *O. collaris* with that found in four codistributed arctic-alpine small mammals in a comparative phylogeographic context

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Chapter 1:

Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques[1]

1.1 Abstract

Although several studies have recently addressed phylogenetic relationships among Asian pikas (*Ochotona* spp.), the North American species have been relatively neglected and their monophyly generally unquestioned or assumed. Given the high degree of intraspecific diversity in pelage and call structure, the recent identification of previously unrecognized species of pika in Asia, and the increasing evidence for multiple trans-Beringian dispersals in several small mammal species, the monophyly of North American pikas warrants reexamination. In addition, previous studies have applied an externally calibrated rate to examine the timing of diversification within the genus. This method has been increasingly shown to return results that, at the very least, are overly narrow in their confidence intervals, and at the worst can be entirely spurious. For this study we combined GenBank sequences from the mitochondrial genes cyt *b* and ND4 with newly generated sequence data from *O. hyperborea* and *O. collaris* to investigate the origin of the North American lineages and the timing of phylogenetic diversification within the genus *Ochotona*. Specifically, we address three goals: (1) summarize and reanalyze the molecular evidence for relationships within the genus using statistically supported models of evolution; (2) add additional sequences from *O. collaris* and *O. hyperborea* to rigorously test the monophyly of North American pikas; and, (3) examine the timing

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[1] Lanier, H.C. and L.E. Olson. 2009. Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques. *Molecular Phylogenetics and Evolution. 53(1): 1-12.*

of the diversification within the genus using relaxed molecular clock methods. We found no evidence of multiple trans-Beringian dispersals into North America, thereby supporting the traditional hypothesis of a single invasion of North America. We also provide evidence that the major splits within the genus occurred in the Miocene, and the Nearctic pikas diverged sometime before the Pleistocene.

1.2 Introduction

Pikas (Lagomorpha: Ochotonidae; *Ochotona*) comprise 30 species of territorial, small-bodied herbivores currently found in the northern hemisphere (Smith et al., 1990; Hoffmann and Smith, 2005). Thought to have diverged from their sister lineage Leporidae (rabbits and hares) sometime after the K/T boundary (65 mya; but see Bininda-Emonds et al., 2007), pikas are one of the least speciose of any of the families in the superorder Glires (Orders Lagomorpha and Rodentia). Pikas are generalist herbivores, adapted to steppe and alpine environments. Extant species (genus *Ochotona*) exhibit high intraspecific variation in pelage and vocalizations but few differences between species (Corbet, 1978; Smith et al., 1990; Erbajeva, 1994). Consequently, their taxonomy has been, and continues to be, poorly resolved (Hoffmann and Smith, 2005).

Two recent molecular studies have explored phylogenetic relationships within *Ochotona* using DNA sequence data. The first (Yu et al., 2000) sampled two mitochondrial genes (1383 bp of ND4 and 1140 bp of cytochrome *b*) from 19 extant species, focusing mainly on the timing and divergence of Asian *Ochotona*. Yu et al. (2000) recovered three main subgroups of pika: a northern subgroup, a shrub-steppe dwelling subgroup, and a mountain subgroup. Largely based on this work, *Ochotona* was grouped into three subgenera (Table 1) by Hoffmann and Smith (2005). While the Yu et al. (2000) analysis provides an important foundation for understanding pika systematics, it included very few intraspecific samples from broadly separated localities and failed to include a number of potentially important species, particularly *O. collaris* (North America) and *O. pusilla* (Eurasia). A second molecular study was recently conducted by Niu et al. (2004), incorporating more taxa but shorter fragments of cyt *b* (~402 bp). As ochotonid taxonomy can be complex and species often misidentified (Smith et al., 1990), the inclusion of additional species and intraspecific samples is important. However, larger

taxonomic sampling frequently requires larger molecular datasets to provide sufficient phylogenetic resolution. Recent morphological (Lissovsky, 2003) and molecular (Lissovsky et al., 2007) studies have also been conducted on the Palearctic members of the subgenus *Pika*. Phylogenetic analyses by Lissovsky et al. (2007) incorporated greater sampling within the *O. alpina-hyperborea* complex, resulting in the recognition of the morphologically, ecologically, and genetically distinct species, *O. turuchanensis*. Lissovsky (2003) and Lissovsky et al. (2007) also suggested the presence of at least one additional species, *O. scorodumovi*, which may be conspecific with *O. mantchurica*.

1.1.1 Nearctic pikas

In North America, two allopatric species of pika are currently recognized—*O. collaris* and *O. princeps*—which have been traditionally defined on the basis of geographic isolation, size of auditory bullae, and pelage characteristics (Nelson, 1893; Hall, 1951; Broadbooks, 1965; MacDonald and Jones, 1987). The many similarities between the two species led some authors to synonymize *O. collaris* and *O. princeps*, along with the Palearctic *O. hyperborea*, with the Palearctic species *O. alpina* (e.g., Broadbooks, 1965; Corbet, 1978). Weston (1981) used morphometric data to show distinct differences between the North American taxa, with *O. princeps* more closely resembling the Asian species. In terms of intraspecific variation, *O. princeps* includes 36 recognized subspecies and a much patchier distribution then the monotypic *O. collaris* (Hall, 1981). This may be an artifact of disproportionate research efforts over the past century, as *O. princeps* has been more frequently studied than *O. collaris* (MacDonald and Jones, 1987; Smith and Weston, 1990). While subspecific differentiation within *O. collaris* has been suggested (Baker, 1951), it has yet to be thoroughly investigated. It may also reflect true levels of intraspecific variation, possibly resulting from events of historical population fragmentation within *O. princeps* or a more recent bottleneck within *O. collaris* (Hafner, 1994; Hafner and Sullivan, 1995).

1.1.2 Biogeography and fossil history

Currently, pika diversity is highest in Asia (28 species), with only two species in North America. The current distribution and diversity of the pikas is a fraction of that seen during their peak in the Miocene, with fossils representing multiple genera known from localities as far apart as north Africa, eastern North America, and western Europe (Dawson 1967). Pleistocene records exist for steppe pika (*O. pusilla*) in Great Britain, although this species is now known only from the central Russian steppes and northern Kazakhstan (Erbajeva, 1994; Fisher and Yalden, 2004; Smith et al., 1990).

Pikas are known from North American localities as early as the Miocene (in Oregon; Shotwell, 1956), and as far south as California, as far east as Virginia in the Pleistocene (Kurtén and Anderson, 1980; Erbajeva, 1994; Mead and Grady, 1996). Several North American species have been described, including a relatively large form (*O. whartoni*) from Alaska and the Yukon Territory (Mead, 1987; Guthrie and Matthews, 1971). A smaller species, possibly *O. collaris*, is thought to have been sympatric (although perhaps not contemporary) with *O. whartoni* (now extinct; Guthrie and Matthews, 1971; Harington, 1978; Weston, 1981; Mead, 1987). Distribution maps of *O. princeps* and *O. collaris* have been used to suggest allopatric speciation in separate refugia during the Wisconsinan glaciation (Guthrie, 1973; Harington, 1978). This scenario would imply that *O. collaris* and *O. princeps* diverged within North America after a single dispersal across the Bering Land Bridge from Asia. However, similar assumptions about the Alaska marmot (*Marmota broweri*) and the hoary marmot (*M. caligata*), also North American alpine specialists with similarly allopatric distributions, proved incorrect, as molecular data suggest that the Alaskan marmot is the result of an independent colonization and is actually more closely related to Asian species (Steppan et al., 1999).

1.1.3 Current objectives

Pikas exhibit a great deal of intraspecific morphological diversity and relatively low interspecific diversity, making it unreasonable to use a single sample per putative species as a proxy for that species. An increasing number of studies have demonstrated the importance of sampling multiple individuals from multiple species to understand the evolutionary history and taxonomic limits of a species (Peters et al., 2005). Using a single individual, or a geographically clumped sample from multiple individuals, could fail to capture paraphyly or polyphyly resulting from introgression, hybridization, and/or incomplete lineage sorting of ancestral polymorphisms (Peters et al., 2007). As many talus-dwelling species live in patchily distributed habitat with disjunct populations (Smith et al., 1990), they may likely contain cryptic lineages. Several of the most recently described species of pika are talus-dwelling, and very geographically restricted (e.g., *O. iliensis*, Li and Smith, 2005; *O. argentata*, Erbajeva and Ma, 2006). Little is known about the potential for pika species to hybridize, but rabbits and hares show evidence of introgression or hybridization between species (Alves et al., 2003). While denser taxonomic sampling is important, it necessitates increasing the number of characters to improve resolution and nodal support (Jansa et al., 2006).

Statistical and computational phylogenetic methods have greatly improved over the past few years, and we sought to utilize those advances to better test unresolved issues in pika phylogeny. Neither the Yu et al. (2000) nor the Niu et al. (2004) study employed a statistically supported model of evolution. Simpler models are known to perform poorly on trees with short internodes and relatively long external branches (Felsenstein, 1978). In addition, they are likely to underestimate the amount of evolution that has occurred on a tree and subsequently bias the branch lengths downward (Yang, 2006). Explicit models utilize parameters that can be captured and compared through hierarchical likelihood ratio tests (hLRT), the Akaike Information Criterion (AIC), or the

Bayesian Information Criterion (BIC) in order to choose the model that best fits the data (Posada and Crandall, 1998; Posada and Buckley, 2004). Accounting for rate variation between sites is critical to correctly estimating branch lengths, and correctly estimating branch lengths is essential for accurately inferring time since divergence.

Many systematic studies construct phylogenies and apply molecular clocks to their data without checking for clock-like behavior in their datasets (Peterson, 2006). Despite the general consensus that the dates obtained from misusing these techniques are probably somewhat incorrect, authors continue to apply molecular clocks in this fashion because they provide a genealogical comparison to geological events. Applying an externally calibrated rate (e.g., 2% divergence per million years for mtDNA) fails to account for rate variation and, when strictly applied, can vastly underestimate the uncertainty around a particular divergence date (Thorne, pers. comm., HCL 2007.; Pulquéro and Nichols, 2007). While using the fossil record to calibrate nodes within a phylogeny is generally agreed to be the most appropriate method, users need to exercise caution to correctly apply dates and incorporate error estimates (Graur and Martin, 2004; Benton and Donoghue, 2007; Ho, 2007). Relaxed molecular clock techniques provide increasingly sophisticated models for estimating a range of plausible divergence dates (Sanderson, 2002; Drummond et al., 2006).

Two of the previous studies of pika systematics using molecular data have assumed a strict molecular clock (Yu et al., 2000; Niu et al., 2004). Each of these studies calculated point estimates of divergence dates between some taxa, most of which fall within the Pleistocene. Several studies (Hoberg, 2005; Waltari et al., 2007) have used dates based on these studies, so we are particularly interested in determining their validity. Rather than applying a strict molecular clock or calibrating with a single point estimate, we are interested in determining the range of plausible divergence dates supported by the data to test the hypothesized

Pleistocene divergence of North American pikas (proposed by Guthrie, 1973). This hypothesized Wisconsinan split in the Nearctic pikas has been cited by many authors (e.g., Harington, 1978; Weston, 1981; MacDonald and Jones, 1987) and is consistent with the divergence dates estimated by Niu et al. (2004), who applied a strict molecular clock. An increasing number of studies are finding that Pleistocene climate changes caused within-species phylogeographic structure but that most speciation events predate the period (Near et al., 2003).

1.2 Methods

1.2.1 Taxon and marker selection

We combined sequences from eight published studies with additional sequences generated specifically for this study to better understand the origin of the Nearctic pikas and the phylogenetic relationships within *Ochotona*. Our aim was to integrate the additional taxonomic sampling of Niu et al. (2004) and other GenBank contributors, the high degree of intraspecific sampling of Lissovsky et al. (2007), and the longer sequences used by Yu et al. (2000). We augmented the existing GenBank data (101 individuals of *Ochotona*, 2 leporid outgroups; Appendix 1) with additional sequences from pikas on either side of the Bering Strait (Appendix 2). We placed particular emphasis on including samples of *O. collaris* from throughout its range, and samples of *O. hyperborea* from localities closest to the Bering Strait.

We analyzed two complementary datasets, one with greater taxon sampling (cyt *b*) and one with fewer taxa but more characters (cyt *b* + ND4). For the cyt *b* dataset, we included most available GenBank *Ochotona* sequences, including several that have recently been called into question. Lissovsky et al. (2007) highlighted potential errors in some published cyt *b* data and, for that reason as well as concerns over specimen misidentification, excluded all sequences generated by Niu et al. (2004). We chose to include the sequences from Niu et al. (2004) because we feel that their analysis in a phylogenetic framework provides important information.

Lissovsky et al. (2007) also excluded an unspecified 153 bp region of another sequence because it contained several unique amino acids (*O. alpina*; GenBank accession AF273009; Yu et al., 2000). While we agree that there are suspicious differences in this particular sequence, a lack of detailed amplification and sequencing strategy (in Yu et al., 2000) meant that we were unable to rigorously test for potential chimerism. However, BLAST results for the general region of AF273009 questioned by Lissovsky et al. (2007) suggested an *Ochotona* origin (results not shown). Additional corroboration comes from an identical haplotype (to AF273009) recovered by Niu et al. (2004) from a different specimen of the same species. Conversely, an *O. pallasi* sequence (DQ335521) published on GenBank by Lissovsky et al. (2007), but inexplicably excluded from their published paper, was excluded from this analysis because a BLAST search returned no similar *Ochotona* sequences.

1.2.2 DNA amplification and sequencing

DNA was extracted from frozen tissues using the PureGene kit (Gentra Systems, Inc.) following the manufacturer's Animal Tissue Protocol. Purified DNA was resuspended in 100 μL DNA Hydration Solution, and a 1:10 working dilution was used in PCR reactions. Amplification followed standard PCR protocols at a magnesium concentration of 1.5 mM, with 30 cycles of denaturation, annealing (50º C), and extension (using primers marked with an asterisk in Table 2). Primers were designed with reference to the complete mitochondrial genomes of *O. collaris* (AF348080) and *O. princeps* (AJ537415). PCR products were purified with Exo/SAP (USB Corp.) and sequenced directly with amplification (external) primers and internal primers and 0.5 μL of ABI BigDye v3.1 terminators (Applied Biosystems) on an ABI 3100 automated sequencer. Double-stranded sequences were generated for all samples, with at least 50 bp of overlap between adjacent fragments. Sequences were assembled and edited using Sequencher 4.5 (GeneCodes Corp.). All sequences

generated in this analysis, along with their specimen voucher numbers, have been deposited in GenBank (accession nos. EU549736-EU549756; Appendix 2).

1.2.3 Phylogenetic analysis

GenBank sequences varied in length from 317-1140 bp and from 820-1383 bp (cyt *b* and ND4, respectively; Appendix 1). Sequences generated specifically for this study were complete (1140 bp for cyt *b* and 1383 bp of ND4 after primer sequences were excised). We aligned sequences by eye in MacClade (Maddison and Maddison, 2000). Phylogenetic analyses were first conducted on each gene separately for data exploration purposes. However, only results for cyt *b* and combined gene (cyt *b* + ND4) analyses are presented and discussed.

We used GARLI (v. 0.95, Zwickl, 2006) to conduct heuristic tree searches and bootstrapping under the maximum likelihood criterion. GARLI employs a genetic algorithm to find the topology and parameters compatible with the data that yield the highest likelihood. Nucleotide substitution models were chosen using the Akaike Information Criterion in ModelTest 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). Analyses were conducted using the model of nucleotide substitution suggested by ModelTest, allowing the parameters within that model to be estimated by GARLI, and using an initial starting topology created using the neighbor-joining method in PAUP* (Swofford, 2002). Likelihood searches were run using the default parameters for run termination and optimization. Each bootstrap pseudoreplicate was run until $-\ln L$ values converged (changing less than 0.02) for 5000 generations. PAUP* was then used to calculate nodal support by computing a consensus tree of the 100 bootstrap pseudoreplicates.

Bayesian posterior probabilities and topologies were estimated using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each single-locus dataset (cyt *b* and ND4), we performed separate Bayesian analyses (BA), one in which parameters were applied to the whole gene with a proportion of invariant sites and a gamma rate distribution. Combined gene datasets were

analyzed using a GTR + I + Γ model, partitioned between genes, and a GTR model partitioned by codons. A Bayes factor comparison showed that for the combined datasets the codon-partitioned model was decidedly better (K = 457; 1000 –ln *L* units) than the gene-partitioned model, and only the results of the codon-partitioned model will be discussed. Tree searches were conducted using four chains, three of which were heated, allowed to run for 10 million generations and sampled every 1,000 generations. The first 200 trees (representing 200,000 generations) were discarded as burn-in, using the plots of –ln *L* scores against generation as a guide.

1.2.4 Molecular clock calibration

While there are a fair number of fossil ochotonids (including but not limited to fossils of extant taxa listed in Table 1), the phylogenetic placement of most fossil taxa within the genus *Ochotona* is uncertain. There are no clear morphological synapomorphies uniting subgenera within *Ochotona* (Yu et al., 2000; Hoffmann and Smith, 2005). However, there are some fairly accurate estimates that have been proposed for the divergence between Leporidae and Ochotonidae. Dates for this node vary depending on whether they are based on fossil evidence alone or molecular trees calibrated with multiple fossil taxa (e.g., McKenna and Bell, 1997 vs. Bininda-Emonds, 2007). Fossil evidence alone suggests that the divergence between Leporidae and Ochotonidae occurred at or before 37 mya (based upon the first fossil occurrence of Ochotonidae in the late Eocene; McKenna and Bell, 1997). The 37 mya divergence also coincides with recent fossil- and molecular-based divergence estimates within Glires (Asher et al., 2005). We chose to contrast the divergence dates within *Ochotona* using a relaxed molecular clock method and a 37 mya leporid-ochotonid split with those estimated under alternative models of rabbit-pika divergence. This method produces a reasonable range of plausible divergence dates for each node within *Ochotona* and excludes those dates that are not compatible with any of the likely rabbit-pika divergences. One of the most recent

dates proposed for the leporid-ochotonid divergence is around 31 mya (inferred using multiple fossil calibrations, nuclear DNA, and a relaxed molecular clock approach; Matthee et al., 2004). While this date appears to contradict fossil evidence, we feel that it provides a lower bound of estimates for hypothesis testing to account for potential taxonomic and stratigraphic errors in calibration. On the other extreme, a recent study employing a supertree approach and multiple fossil calibration points suggests the leporid-ochotonid split may have occurred at or before the K/T boundary (60–70 mya; Bininda-Emonds et al., 2007). This 65 mya model provides an upper bound, encompassing dates resulting from an Early Eocene rabbit-pika split as has been suggested by recently described fossil Lagomorpha (Rose et al., 2008) and other molecular studies (Springer et al., 2003). Instead of choosing one of these dates, or combining all three into one uniform (and fairly noninformative) prior, we conducted three separate analyses using a different calibration point for the root node (31, 37, and 65 mya) to determine the range of plausible divergence dates supported by the data for the major splits within *Ochotona*.

1.2.5 Molecular dating methods

We used a relaxed molecular phylogenetics technique, implemented in the program BEAST (Drummond et al., 2006), to simultaneously estimate phylogeny and divergence times within *Ochotona*. For the purposes of estimating divergence dates, we analyzed a pruned version of the combined gene dataset that minimized the number of intraspecific samples (see Appendices 1 and 2). Population-level samples are undergoing mutation/fixation at different rates from species-level samples and tend to have very short branches that complicate rate estimation (Ho et al., 2005). BEAST runs were conducted using a GTR model of evolution, with the dataset partitioned by codons and rates unlinked between partitions. We used a relaxed lognormal molecular clock model, which has been shown to generate accurate estimates of rates with narrow Highest Posterior Density intervals

(Drummond et al., 2006; Ho, 2007). We used a Yule prior on the tree to simulate the process of speciation. Operators were tuned with successive runs of the program under the chosen model, with slight changes to the scaling factors until the Effective Sample Size for each parameter exceeded 200 (as recommended by Drummond and Rambaut, 2007). Once optimum operator scaling was achieved, we re-ran the analysis six times (twice for each potential calibration point corresponding to the lagomorph-ochotonid split) for 10 million generations. A 10% burn-in was discarded from the beginning of each run, and all samples were examined in Tracer (Rambaut and Drummond, 2004) to verify an effective sample size exceeding 200 for all parameters being estimated. Independent runs for each calibration point were then combined to yield parameter estimates for divergence dates.

1.3 Results

1.3.1 Model selection and phylogenetic results

ModelTest found that the preferred model of evolution for the cyt *b* dataset, according to both the hierarchical likelihood ratio test and the AIC, is the General Time Reversible model (GTR) with a gamma-distributed shape parameter (α = 1.0656) and a proportion of invariant sites (I = 0.5199). The GTR + Γ + I was also selected for the ND4 matrix (α = 1.1584; I = 0.4772) and the combined matrix (α = 1.1481; I = 0.5077).

When all available cyt *b* samples for *Ochotona* were analyzed, we generally recovered monophyly of the three main subgenera found by Yu et al. (2000) (Fig. 1), with high support (74 ML BS, 0.94 posterior probability) for subgenus *Pika* and moderate to low support for the other subgenera. For the combined dataset, topologies from all analyses were the same, or non-conflicting. The placement of the root was variable between analyses. While the Bayesian cyt *b* + ND4 analyses rooted between the *Conothoa* and *Pika* + *Ochotona* clades, the ML topology resulted in an alternative rooting on the branch leading to *O. erythrotis* at the base of *Conothoa* (Fig

2, black dot). The cyt *b* tree tends to root on *O. pusilla*, with *O. huangensis* weakly supported as being basal to the *Pika + Conothoa* clade, but there was some alternative support for rooting within *Conothoa* (Fig. 1, black dots). Subgenera *Ochotona* and *Pika* (Yu et al.'s shrub-steppe and northern subgroups, respectively) were strongly supported (85% BS, 99% PP) as being sister to one another in all cyt *b* + ND4 analyses. When the cyt *b* dataset is analyzed, the relative placement of the subgenera is not well resolved.

Under most algorithms and models (partitioned by codon and unpartitioned), the placement of *O. pusilla* is not well resolved (Fig. 1). In some cases it is recovered as being basal, and therefore outside the three described subgenera, but this relationship never received strong (>70 posterior probability or bootstrap) support. The placement of *O. huangensis* is poorly resolved in all reconstructions. While it was (54% BS, 84 PP) recovered as sister to the shrub-steppe group (subgenus *Ochotona*) in the combined dataset , it was weakly supported as sister to *O. pusilla* in the maximum likelihood tree (<50 BS, <75 PP). *Ochotona pallasi helanshanensis*, thought to be synonomous with *O. argentata* (Erbajeva and Ma, 2006), is nested within *O. pallasi.* The sequence identified as *O. annectens* (AF273008; Yu et al., 2000) groups most closely with several *O. curzoniae* sequences (Fig. 1) and is not supported as being closely allied to *O. dauurica* despite its putative subspecific status (*O. dauurica annectens;* Hoffmann and Smith, 2005). The questionable *O. alpina* sequences (starred in Fig. 1) were recovered on a particularly long branch as being sister to the rest of *O. alpina.*

Out of the 15 species for which more than one sample per species was included, 10 were recovered as monophyletic. The remaining five were not recovered as monophyletic in any of our analyses. Most of these were within the subgenus *Ochotona* and involved burrowing species. Within two species, *O. princeps* and *O. hyperborea*, relatively deep intraspecific divergences were inferred, comparable to or exceeding divergence between other species of *Ochotona* (as

measured by branch length). Despite the additional samples of *O. hyperborea* and *O. collaris* from either side of the Bering Strait, considerable support was still recovered for the monophyly of the North American taxa. Under all algorithms and all models, *O. collaris* was strongly supported as the sister to *O. princeps*, and the previously recovered relationships within the subgenus *Pika* (*O. pallasi* sister to the *alpina-hyperborea* complex) were unequivocally supported in all analyses.

1.3.2 Molecular dating

All three models of leporid-ochotonid divergence supported a Miocene common ancestor for the extant *Ochotona* (Table 3). While both the 31 and 37 mya models supported Middle to Late Miocene subgeneric diversification, the 65 mya model extends this to the Early to Middle Miocene. Substitution rates were likewise scaled depending on the timing of the leporid-ochotonid split, with the 31 mya model favoring a rate ranging from 1.7-2% million years, the 37 mya root model suggested a rate of 1.4-1.7% million years, and a rate of 0.2-1% mya under the 65 mya model. The older the node, the greater the discrepancies between dates inferred under a strict molecular clock and those resulting from a relaxed molecular clock approach (Fig. 4).

In both the 31 and 37 mya scenarios, the major diversifications within the subgenus *Conothoa* were Late Miocene to Early Pliocene (Fig. 2). These models supported a more recent common ancestor (MRCA) for most of the members of the subgenus *Ochotona*, with the majority of the support being for a Pliocene or Pleistocene diversification. The 65 mya scenario places most of the major divergences within the genus *Ochotona* during the Miocene, with some of the 95% credibility intervals (CI) for the MRCA of the genus occurring in the late Oligocene to early Miocene. None of the three analyses recovered support (within the 95% CI set of trees) that included a common ancestor of *O. collaris* and *O. princeps* in the Pleistocene (Table 3; Fig. 3).

1.4 Discussion

1.4.1 Model selection, branch lengths, and taxonomic implications

In general, incorporating a statistically supported model of evolution resulted in changes in branch length, with topological changes resulting around long branches with short internodes (e.g., within subgenus *Conothoa*). In their maximum parsimony tree, Niu et al. (2004) recovered strong bootstrap support for an *O. alpina* + *O. pallasi* clade, which was not recovered using any other methods in their paper or by other authors (e.g., Yu et al., 2000; Lissovsky et al., 2007; this study). We believe this to be the result of long-branch attraction, and were able to replicate their results using a dataset trimmed to the same 402 bp and subjected to parsimony analysis (results not shown). This example highlights the need for the use of both appropriate models of evolution and large numbers of characters. Incorporating a more statistically supported, and hopefully correct, model of sequence evolution is also particularly important in cases where timing of events is being tested or even discussed. Trees incorporating these models were more than twice as long as those relying on more simplistic models (Yu et al., 2000; Niu et al. 2004). Although these models make more assumptions, we feel that their use is statistically justified through higher likelihood scores and empirically justified by the high probability of multiple changes to the same nucleotide in the mitochondrial genome (Posada and Crandall, 1998; Posada and Buckley, 2004).

The placement of the root is variable, affecting phylogenetic support for the inclusion of two taxa (*O. pusilla* and *O. huangensis*) in the subgenus *Ochotona*, where they were placed by Hoffmann and Smith (2005). The position of the root in our three datasets affects the interpretation of phylogenetic results, and we feel that much work remains to determine species limits and phylogenetic relationships within *Ochotona*. Pleistocene fossils, a broad distribution, and a karyotype of 2N = 68 (identical to the North American taxa) have all been used to justify the basal position of this taxon within the pikas (Erbajeva, 1994; Niu et al., 2004). However,

O. pusilla has only ever been weakly supported as sister to the other *Ochotona*. Further detailed studies, with more genetic data, are necessary to determine the correct placement of the taxon.

1.4.2 Non-monophyly of nominal species

Recent molecular studies of *Ochotona* have provided important insights into relationships within the genus. They have also provided a large number of conflicting sequences that indicate a need for future phylogenetic assessments and taxonomic delimitation. In one-third of the cases where multiple sequences were available for a nominal species, that species was not recovered as monophyletic. There are multiple possible reasons for the non-monophyly of these taxa, including specimen misidentification and/or mtDNA introgression between species. This highlights the need to associate sequences with voucher specimens that have stable museum catalog numbers, both in papers and in GenBank submissions (Ruedas et al., 2000; Peterson et al., 2007). Comprehensive molecular and morphological analyses need to be done to further delimit species within *Ochotona*. This also suggests that until species can be better delimited within the group, comparative phylogenetic analyses need to verify both morphological species identity and mtDNA haplotypes for their results to be repeatable and easily interpreted. Recent studies (Yang et al., 2008) have provided interesting and compelling evidence of adaptive evolution at the protein level within *Ochotona*, and better phylogenetic resolution would almost certainly allow researchers to address and rigorously test more complex hypotheses.

The Northern pika (*O. hyperborea*) and the American pika (*O. princeps*) have long been considered to be two of the most morphologically variable pika species (Smith et al., 1990; Smith and Weston, 1990; Lissovsky, 2003), with 11 and 36 subspecies described, respectively. On a molecular basis, these two species are also some of the most divergent, with intraspecific levels of divergence comparable to or greater than those observed between other species. This coincides with Hafner and

Sullivan's (1995) findings of high degrees of genetic differentiation between populations of *O. princeps* resulting from a history of population fragmentation. The incorporation of additional samples of *O. hyperborea* increased the support for the main geographic clades of *O. hyperborea* described by Lissovsky et al. (2007). In contrast, relatively little genetic divergence is evident in *O. collaris*, suggesting a recent population expansion in this species, similar to that inferred for *O. turuchanensis.* While the latitudinal contrasts in climate and glaciation may explain the differences between geographic structuring in *O. collaris* and *O. princeps* (Hafner, 1994), it does less to explain the differences between *O. hyperborea* and *O. collaris.*

1.4.3 Nearctic pikas diverged before the Pleistocene

Monophyly of the North American pikas was recovered in all phylogenetic analyses. While we are not the first to recover this relationship, our inclusion of multiple samples from throughout the range of both *O. hyperborea* and *O. collaris* makes this the most rigorous test of Nearctic pika monophyly to date. As the addition of multiple intraspecific samples results in non-monophyly for a number of other species of pikas, we felt that this represents significant and meaningful support for the monophyly of the North American taxa. Given the large number of Pleistocene trans-Beringian exchanges observed in other mammalian taxa (Steppan et al., 1999, Galbreath and Cook, 2004; Waltari et al., 2007), the inclusion of these additional individuals is necessary to test the monophyly of the extant North American species. Unfortunately, because *O. collaris* and *O. princeps* are reciprocally monophyletic, these data tell us nothing about the direction of colonization (i.e., whether *O. collaris* represents a separate northern invasion from a southern Nearctic ancestor shared with *O. princeps*).

The oldest record of North American *Ochotona* is *O. spanglei*, known from the Miocene in Oregon (Shotwell, 1956). Fossil evidence has been used to suggest that *O. collaris* and *O. princeps* shared a common ancestor that migrated from Asia in the early Pleistocene or late Pliocene, representing a second or third trans-Beringian

dispersal event for pikas (Erbajeva, 1994), and diverged during the Pleistocene (Guthrie, 1973). *Ochotona whartoni* and a smaller species of pika (presumably *O. collaris* or *O. princeps*) were present in the same sites in the Yukon Territory during the Pleistocene (Harington, 1978; Weston, 1981) at least as early as the Sangamon interglacial (0.130-0.115 mya, Marine Isotope Stage 5e). Our data support an earlier divergence between the Nearctic and Palearctic lineages, as well as an earlier split between the two lineages of extant Nearctic species (*contra* Guthrie, 1973, and Niu et al., 2004). While we cannot exclude the possibility that the lineages that became *O. collaris* and *O. princeps* diverged in Asia and dispersed to North America separately, the most biogeographically parsimonious explanation is that one common ancestor crossed the Bering Land Bridge and subsequently diverged.

It is generally suggested that *Ochotona* invaded North America twice, once by *O. spanglei* and once by the *O. princeps-collaris* lineage. All three scenarios (31, 37, and 65 mya roots) indicate a sufficiently old Nearctic split as to accommodate an *O. spanglei* ancestor. Our results suggest that the Nearctic pikas shared a most recent common ancestor with the Palearctic clade in the subgenus *Pika* between 4.7 and 15.7 million years ago (depending on the calibration of the leporid-ochotonid split).

1.4.4 Relaxed molecular clocks and speciation in *Ochotona*

Despite the broad swath of time represented by each model, there are some biogeographic and evolutionary conclusions that can be drawn from our results. The middle Miocene to early Pliocene was considered to be the 'acme of development' for pikas (Dawson, 1967; Erbajeva, 1994), and most of the basal splits on our calibrated trees fall within this period. *Ochotona pusilla* is considered to be a late Pliocene relic based on dental morphology, karyotype, and a relatively old fossil history (Erbajeva, 1994). If the weakly supported rooting of the tree on *O. pusilla* is correct, this species would have shared a common ancestor with the other *Ochotona* at some point prior to the date estimated for the root (Table 3; Fig. 2, Node A). The antiquity of this lineage is supported by fossil *O. pusilla* from the Pliocene (Table 1).

The subgenera *Conothoa* and *Pika* speciated quickly, probably during the late Miocene to early Pliocene, and represent some of the oldest surviving lineages within the genus. This contrasts sharply with our findings for the subgenus *Ochotona*, where most speciation events date to the Pliocene and Pleistocene. This may be related to their differing ecotypes, as both *Conothoa* and *Pika* are primarily composed of talus-dwelling species. The low fecundity and low dispersal capability of talus-dwelling pikas may predispose them to vicariant speciation with little competition between incipient species. Species in the subgenus *Ochotona* are mostly burrowing (Smith et al. 1990). Burrowing pikas tend to have larger, more frequent litters and undergo greater population fluctuations (Smith, 1988). Their evolutionary history may have therefore been more influenced by rapid replacement between competing lineages, resulting in fewer surviving lineages relative to talus-dwelling species.

Perhaps the most novel contribution of this study is the application of rigorous hypothesis testing to evaluate alternative scenarios of diversification within a genus. Strict application of molecular clocks, particularly those using externally calibrated rates, can lead to overly narrow, and frequently incorrect, point estimates of divergence dates (Pulquéro and Nichols, 2007; Ho, 2007; Fig. 4). Even given the advantages offered by relaxed molecular clock techniques, incorrect fossil calibrations can still result in incorrect dates (Benton and Donohue, 2007; Fig. 3). However, even lacking a series of well-supported fossil calibrations, specific hypotheses may still be testable. We recovered no support for a Pleistocene common ancestor for Nearctic pikas. Similarly, none of the divergences estimated from strict molecular clock approaches were supported under any of our models (Fig. 4). While the broad sets of credibility intervals for each node encompass a large span of time, and are thus more difficult to correlate to particular geologic and climatic events, they may be more representative of biological reality and the tempo of speciation.

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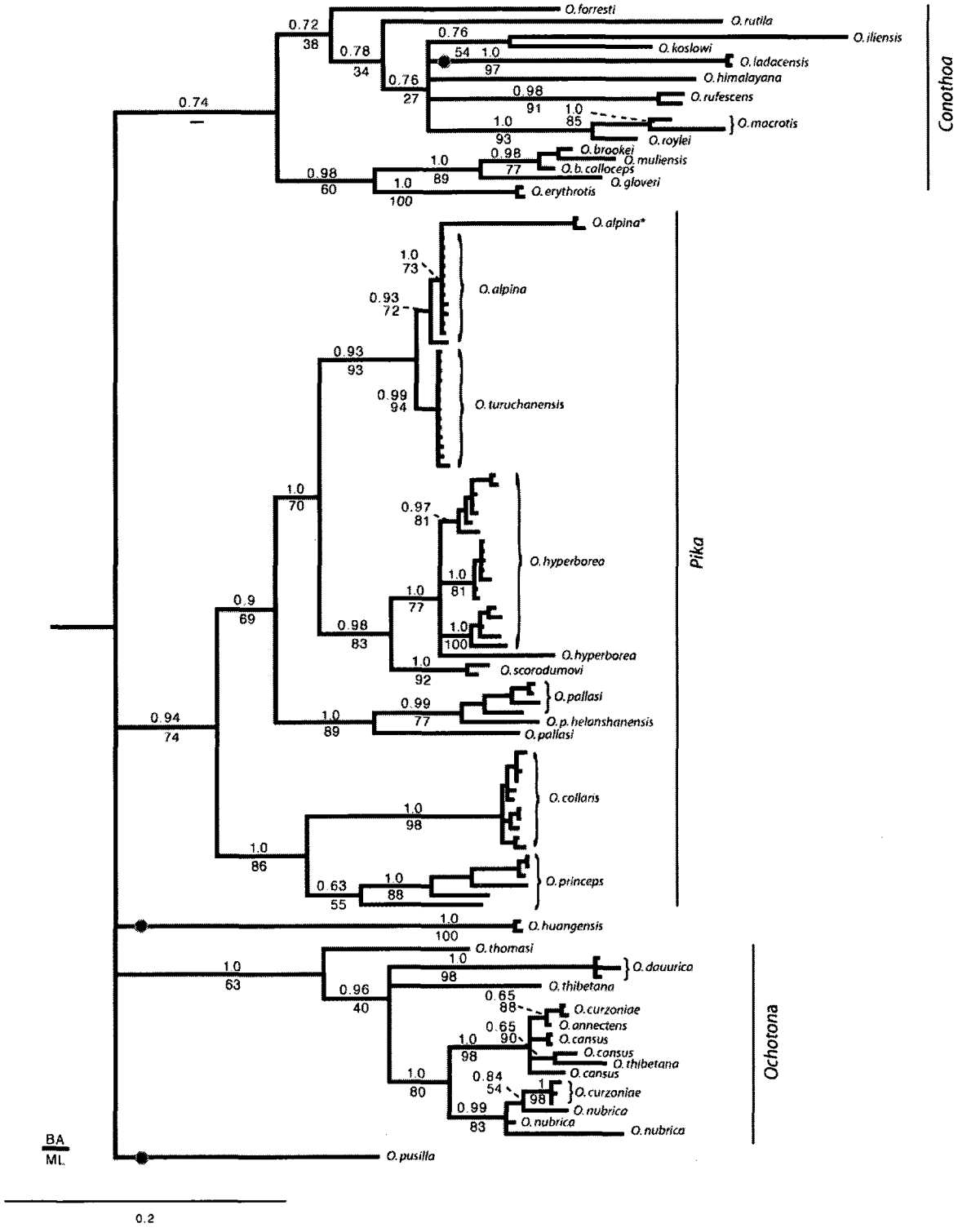
Fig. 1 – Consensus tree obtained from phylogenetic analysis of cyt *b* sequences using MrBayes. Nodal support indicated by Bayesian posterior probabilities (BA, top) and ML bootstrap values, (ML, bottom). Support not shown for terminal nodes between conspecific taxa. Subgeneric assignments from Hoffmann and Smith (2005) are shown where subgenera are monophyletic. Black dots indicate variable position of root (see text).

1.6 Figures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O. rutila
O. iliensis
O. koslowi
O. ladacensis
O. himalayana
O. rufescens
O. macrotis
O. roylei
O. brookei
O. muliensis
O. b. colloceps
O. gloveri
O. erythrotis
Conothoa
O. alpina*
O. alpina
O. turuchanensis
O. hyperborea
O. hyperborea
O. scorodumovi
O. pallasi
O. p. helanshanensis
O. pallasi
O. collaris
O. princeps
Pika
O. huangensis
O. thomasi
O. dauurica
O. thibetana
O. curzoniae
O. annectens
O. cansus
O. cansus
O. thibetana
O. cansus
O. curzoniae
O. nubrica
O. nubrica
O. nubrica
Ochotona
O. pusilla
BA
ML
0.2

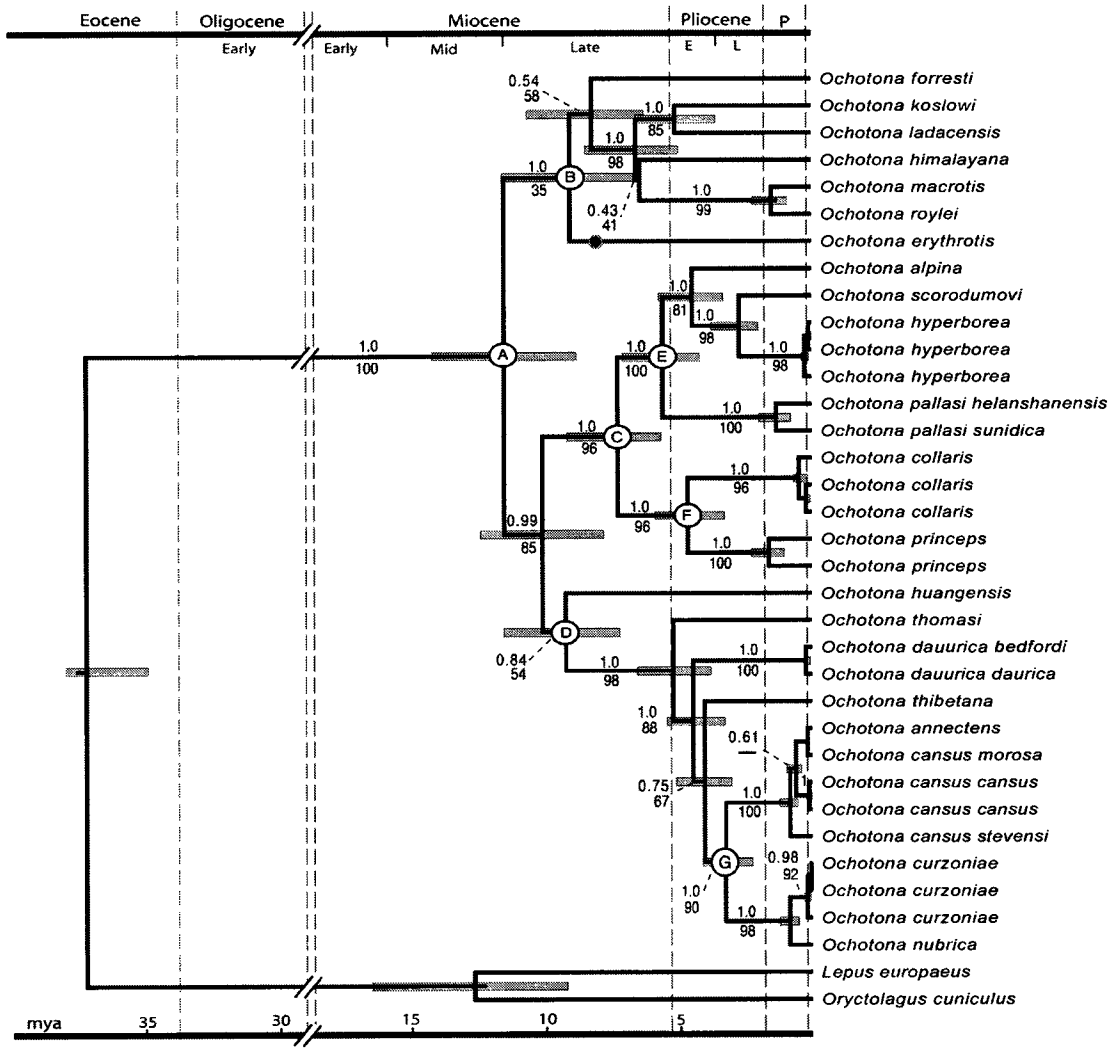

Fig. 2 – Divergence dates and 95% credibility intervals resulting from analysis of the 37 mya rooted model (cyt b + ND4 dataset) in Bayesian relaxed molecular dating technique implemented in BEAST. Nodal support as in Fig.1. Geological time scale follows Gradstein et al. (2004).

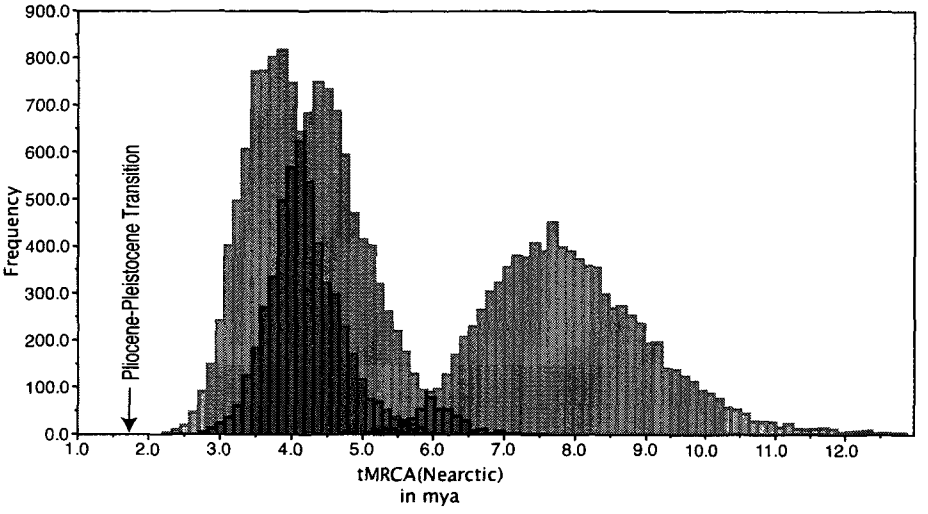

Fig. 3 – Range of posterior estimates for tMRCA (time to most recent common ancestor) of Nearctic pikas; divergence dates estimated under the 31, 37, and 65 mya root models (left to right).

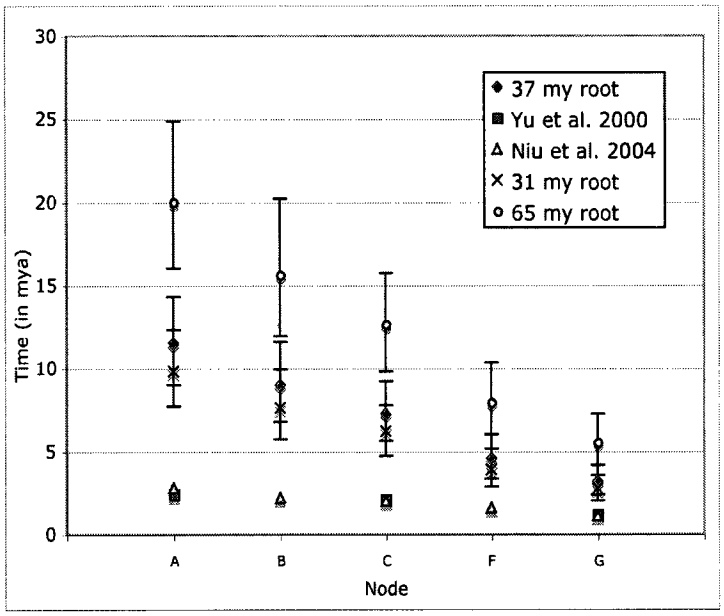

Fig. 4 – Comparison of nodal dates estimated under this study with those reported in previous analyses (Yu et al., 2000; Niu et al., 2004). Nodes correspond to those shown in Figure 2 and Table 3. Dates resulting from other studies are aligned with columns to which they correspond, but horizontally spaced to show 95% credibility intervals.

1.7 Tables

Table 1 – Subgenera and fossil record of extant *Ochotona* species

Subgenus[1]	Species	First known fossil occurrence
Conothoa	*O. brookei*	
	O. erythrotis	Late Pliocene[2]
	O. forresti	
	O. gaoligongensis	
	O. gloveri	
	O. himalayana	
	O. iliensis	
	O. koslowi	Pleistocene[3]
	O. ladacensis	
	O. macrotis	Middle Pleistocene[4]
	O. muliensis	
	O. nigritia	
	O. roylei	
	O. rutilla	
Ochotona	*O. cansus*	
	O. curzoniae	
	O. dauurica	Middle Pleistocene[5]
	O. huangensis	
	O. nubrica	
	O. pusilla	Late Pliocene[5]
	O. rufescens	Middle Pleistocene[5]
	O. thibetana	Late Pleistocene[3]
	O. thomasi	
Pika	*O. alpina*	Middle Pleistocene[5]
	O. argentata	
	O. collaris	Late Pleistocene[6]
	O. hoffmanni	
	O. hyperborea	Late Pleistocene[5]
	O. pallasi	
	O. princeps	Middle Pleistocene[7]
	O. turuchanensis	

[1]Hoffmann and Smith (2005)
[2]Cai (1989);
[3]Li et al., (2006)
[4]Erbajeva and Zheng (2005)
[5]Erbajeva (1994)
[6]Harington (1978)
[7]Mead (1987)

Table 2 – Primers

Name	Sequence
CB-HLF1*	5' - CCACCGTTGTAGTTCAACT
CB-HLiF2	5' - AGCCACCCTAACTCGATTCT
CB-HLiR2	5' - AGCCTGTTTCGTGGAGGAAGAGTA
CB-HLR1*	5' - GGTTTACAAGACCAGGGTA
ND4-HLF1*	5' - CCAACACATACGGCATAGACTA
ND4-HLiF2	5' - ATCACCCGATGAGGTAACCAAACA
ND4-HLiF3	5' - TGGCACTCGTAATTGTCGCAA
ND4-HLiR3	5' - TTGTGGATGTAGAGTAGGGCTA
ND4-HLiR2	5' - TTCGGCTGTGGATACGTTCATA
ND4-HLR1*	5' - CGGTGGATAAGAGGTTGTTA

*external primers

Table 3 – Estimated tMRCA for the major splits within *Ochotona*

	Node	31 Mya Model	37 Mya Model	65 Mya Model
	Root (posterior)	31.641	36.958	64.969
		(29.632-33.527)	(34.952-38.879)	(63.03-66.941)
A	*Ochotona*	9.913	11.626	20.253
		(7.701-12.338)	(8.98-14.317)	(16.013-24.857)
B	*Conothoa*	7.722	9.091	15.971
	'mountain'	(5.703-9.913)	(6.842-11.687)	(11.945-20.221)
C	*Pika*	6.246	7.326	12.775
	'northern'	(4.73-7.77)	(5.63-9.208)	(9.806-15.717)
D	*Ochotona*	7.9878	9.3493	16.277
	'shrub-steppe'	(6.0845-9.897)	(7.2343-11.653)	(12.765-20.2594)
D	Palearctic	4.7774	9.0886	9.7818
		(3.6534-6.0814)	(6.7772-11.6095)	(7.3517-12.1717)
E	Nearctic	3.941	4.621	8.045
		(2.847-5.14)	(3.345-5.984)	(5.998-10.329)

Dates given in millions of years before present, with mean above and 95% HPD below. Letters in first column correspond to nodes indicated on Fig. 2.

1.8 Literature Cited

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1.9 Appendices

Appendix A– GenBank sequences used in this study

Taxon	Cyt b accn	Length	ND4 Accn	Length	Citation
O. alpina	AF273009	1140 bp	AF273130	1383 bp	Yu et al., 2000
	AY056605	402 bp			Niu et al., 2004
O. annectens	AF273008	1140 bp	AF273129	1383 bp	Yu et al., 2000
O. brookei	AY056600	402 bp			Niu et al., 2004
O. brookei calloceps	AY191825	402 bp			Niu et al., 2004
O. cansus cansus	AF273003	1140 bp	AF273125	1383 bp	Yu et al., 2000
	AF273006	1140 bp	AF273126	1383 bp	Yu et al., 2000
O. c. morosa	AF273007	1140 bp	AF273127	1383 bp	Yu et al., 2000
O. c. stevensi	AF273005	1140 bp	AF273128	1383 bp	Yu et al., 2000
O. collaris	AF176578	682 bp			Hafner et al., (unpub)
	AF348080	1140 bp	AF348080	1383 bp	Lin et al., 2002
	AY056608	402 bp			Niu et al., 2004
O. curzoniae	AF176581	682 bp			Hafner et al., (unpub)
	AF273001	1140 bp	AF273122	1383 bp	Yu et al., 2000
	AF273002	1140 bp	AF273123	1383 bp	Yu et al., 2000
	AF273004	1140 bp	AF273124	1383 bp	Yu et al., 2000
	AF432908	402 bp			Niu et al., 2004
O. dauurica bedfordi	AF273000	1140 bp	AF273134	1216 bp	Yu et al., 2000
O. d. dauurica	AF273011	1140 bp	AF273135	1190 bp	Yu et al., 2000
O. erythrotis	AF272999	1140 bp	AF273121	1383 bp	Yu et al., 2000
	AY056606	402 bp			Niu et al., 2004
O. forresti	AF272998	1140 bp	AF273120	1383 bp	Yu et al., 2000
O. gloveri	AY056602	402 bp			Niu et al., 2004
O. himalayana	AF272997	1140 bp	AF273119	1383 bp	Yu et al., 2000
O. huangensis	AF272995	1140 bp	AF273117	1383 bp	Yu et al., 2000
O. h. xunhuaensis	AY191821	402 bp			Niu et al., 2004
O. hyperborea	AB053257	1140 bp			Takaki et al., (unpub)
	AF176582	682 bp			Hafner et al., (unpub)
	AF272994	1140 bp	AF273115	1383 bp	Yu et al., 2000
	AY056603	402 bp			Niu et al., 2004
O. iliensis	AY191824	402 bp			Niu et al., 2004
O. koslowi	AF272993	1140 bp	AF273116	1383 bp	Yu et al., 2000
O. ladacensis	AF272992	1140 bp	AF273114	1383 bp	Yu et al., 2000
	AY056609	402 bp			Niu et al., 2004
O. macrotis	AF273010	1140 bp	AF273133	820 bp	Yu et al., 2000
O. m. macrotis	AY191820	402 bp			Niu et al., 2004
O. muliensis	AF421884	402 bp			Niu et al., 2004
O. nubrica	AF272991	1140 bp	AF273113	1383 bp	Yu et al., 2000
O. n. lama	AY191823	402 bp			Niu et al., 2004
O. n. lhasaensis	AY191822	402 bp			Niu et al., 2004
O, pallasi	AY056607	402 bp			Niu et al., 2004
O. p. helanshanesis[a]	AF272996	1140 bp	AF273118	1383 bp	Yu et al., 2000
O. p. pricei	AY117696	402 bp			Niu et al., 2004
O. p. sunidica	AF272990	1140 bp	AF273132	1218 bp	Yu et al., 2000
O. princeps	AF176579	682 bp			Hafner et al., (unpub)
	AJ537415	1140 bp	AJ537415	1383 bp	Gissi and Pesole (unpub)
	AY056604	402 bp			Niu et al., 2004
	AY292716	1140 bp			Mathee et al., 2004

	U58940	653 bp			Halanych and Robinson, 1999
	AF272989	1140 bp	AF273112	1383 bp	Yu et al., 2000
O. pusilla	AY260744	402 bp			Niu et al., 2004
O. roylei	AF272988	1140 bp	AF273131	1216 bp	Yu et al., 2000
O. rufescens	AJ132206	1140 bp			Barome et al., (unpub)
O. rutila	AF515733	402 bp			Niu et al., 2004
	AY056601	402 bp			Niu et al., 2004
O. thibetana	AF176580	682 bp			Hafner et al., (unpub)
	AF272986	1140 bp	AF273110	1383 bp	Yu et al., 2000
O. thomasi	AF272987	1140 bp	AF273111	1383 bp	Yu et al., 2000

a. Considered to be *O. argentata* by Erbajeva and Ma (2006) on the basis of karyotype, pelage, and size

b. Although *O. scorodumovi* may be synonymous with *O. mantchurica*, we follow the taxonomy used by Lissovsky et al. (2007) to facilitate comparisons or our results with theirs

c. Originally designated as *O. hyperborea* by Yu et al. (2000). We follow the taxonomy of Lissovsky et al. (2007).

Appendix B – Voucher specimens

Species	Voucher	GenBank Accn	Locality
O. collaris	UAM 31645	EU549741[a]	United States, Alaska: Snowhawk Lake
	UAM 35126	EU549747[a] EU549742[b]	United States, Alaska: White Pass; 500 m S of USA/Canada border along railroad
	UAM 57694[c]	EU549748[a] EU549743[b]	United States, Alaska: Pocket Creek
	UAM 58205	EU549749[a] EU549738[b]	United States, Alaska: mountainside NW of headwater lake of Crescent Creek
	UAM 58445	EU549750[a] EU549739[b]	United States, Alaska: E slope of Mt. Kathryn, S of Woodchopper Creek
	UAM 63935	EU549751[a] EU549736[b]	United States, Alaska: Eagle Summit, E side of Steese Hwy on talus slope
	UAM 63936[c]	EU549752[a] EU549740[b]	United States, Alaska: Eagle Summit, E side of Steese Hwy on talus slope
	UAM 71652[c]	EU549753[a] EU549737[b]	Canada, Yukon Territory: Thandlat
O. hyperborea	UAM 23239[c]	EU549754[a] EU549744[b]	Russia, Magadanskaya oblast: Stokovo
	UAM 80090[c]	EU549755[a] EU549746[b]	Russia, Magadanskaya oblast: Contact Station, 150 km NW Ust-Omchug
	UAM 80812[c]	EU549756[a] EU549745[b]	Russia, Magadanskaya oblast: 40 km W Magadan

a. Cyt *b*
b. ND4
c. Used in molecular dating analysis

Chapter 2:

Environmental influences on body size in collared pikas (*Ochotona collaris*)[1]

2.1 Abstract

Aim I examined trends in body size of the collared pika (*Ochotona collaris*) across its range in Alaska and northwest Canada and over the past century to test conformance to Bergmann's rule. Bergmann's rule predicts that larger body size should occur in populations inhabiting colder, higher latitude environments. Contrary to this are predictions resulting from body size gradients driven by resource availability, under which body size will be negatively correlated with latitude in response to the length of the growing season. The purpose of this paper is twofold: first, to test for morphological variation across space and time within *O. collaris*; and second, to examine relationships between climate variables and body size.

Location Alaska and northwestern Canada, North America

Methods I measured 257 museum specimens, representing 110 years of sampling from throughout the distribution of *O. collaris.* Measurements were regressed against collecting variables (latitude, longitude, year of collection, and Julian date) and local and global climatic variables to determine the presence of latitudinal, temporal, and climate-related trends in morphology. I used a model selection approach to evaluate the best predictor variables. In addition, I compared body size metrics for a small sample of pikas from the same population at two different time points to examine change over time where latitude is held constant.

Results

A strong inverse relationship was found between skull size and latitude. Sexual dimorphism in skull size was also evident, with more pronounced dimorphism in

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[1] Lanier, H.C. Environmental influences on body size in collared pikas (*Ochotona collaris*). Prepared for submission to *Journal of Biogeography.*

the southern populations. Skull size increased significantly over the 110-year period and with midwinter temperature anomalies, but was negatively correlated with local summer temperatures.

Main conclusions Skull size of collared pikas follows an inverse-Bergmannian pattern. When considered in conjunction with temporal trends, this suggests that body size is decreasing following the cline in growing season and food availability (the resource availability hypothesis). The negative interaction between local summer temperature and skull size may be indicative of secondary effects of selection for a thermoregulatory optimum.

2.2 Introduction

2.2.1 Climate change and ecogeographic rules

Animal and plant species adapt to changing environments through a combination of behavioral, phenotypic, genetic, and distributional mechanisms (Parmesan & Yohe, 2003; Berteaux *et al.*, 2004; Parmesan, 2006; Moritz *et al.*, 2008). Ecogeographic variations in body size, such as those predicted under Bergmann's rule (*sensu* Rensch, 1938), are often hypothesized to represent adaptations to ecological and/or environmental gradients that may also occur over time (Millien *et al.*, 2006; Gaston *et al.*, 2008). Bergmann (1847; translated in James, 1970) suggested that for homeothermic organisms the limitation of surface area to volume (hereafter surface:volume) ratio plays a role in determining body size; when other factors are equal the smaller *species* in a *genus* will occur in warmer regions. Although the underlying physiological forces invoked under Bergmann's rule have been contentious (e.g., Scholander, 1955), many studies have found support for the overall interspecific pattern (e.g., Blackburn & Hawkins, 2004; Rodríguez *et al.*, 2008). Less contentious is the application of Bergmann's rule at the intraspecific level (Mayr, 1963; James, 1970). Within a species the insulative properties of fur and feathers may vary less, and slight changes to the surface:volume ratio may provide a greater advantage by affecting the rate of dissipative heat loss (Brown and Lee, 1969). As temperature often co-varies with latitude, Bergmann's rule is frequently investigated relative to latitudinal or altitudinal gradients (Rodríguez *et al.*, 2008; Liao *et al.*, 2007). While latitude has often been used as proxy for ambient temperature, it has been suggested that other explanatory factors that co-vary with temperature (e.g., moisture, primary productivity, food and/or habitat availability) may cause observed clinal variation (Rosenweig, 1968; James, 1970; Hawkins & Diniz-Filho, 2004; Rodríguez *et al.*, 2008).

Phenotypic changes, such as variations in body size can occur over decadal timescales and are thought to be some of the first responses to climate change (Barnosky *et al.*, 2003; Smith & Betancourt, 2003; Millien *et al.*, 2006). Recent studies have documented short-term (less than a century) changes in body size within species in response to anthropogenic climate change (e.g., Yom-Tov & Yom-Tov, 2005; Teplitsky *et al.*, 2008; Yom-Tov *et al.*, 2008). However, temporal analyses on carnivore body size, which might be expected to exhibit greater variation as many carnivores follow Bergmann's rule (Meiri *et al.*, 2004), suggest that these trends are not universal (Meiri *et al.*, 2009). Over longer periods of Quaternary climate change, body size has also been shown to vary in extant small mammals (e.g., Smith & Betancourt, 2003; Blois *et al.*, 2008) and prior to extinction in North American caballoid horses (Guthrie, 2003). Arctic and alpine regions are hypothesized to be the ecosystems most strongly affected by climate change (AICA, 2008). Arctic warming has been 2-3 times the global average (Post *et al.*, 2009), resulting in earlier snowmelt (Stone *et al.*, 2002) and a greater abundance of shrubs (Sturm *et al.*, 2001). As a result, we might expect phenotypic and phenological changes to be more pronounced in species that occur at higher northern latitudes.

Collared pikas (*Ochotona collaris*) are small, territorial herbivores found on talus slopes in Alaska and northwestern Canada. Like other lagomorphs, collared pikas do not hibernate; as a result, each individual will spend hours collecting, caching, and defending piles of summer vegetation for overwinter consumption (MacDonald & Jones, 1987). Pikas have high metabolisms and a narrow thermal-neutral zone, which makes them extremely susceptible to summer heat stress (MacArthur & Wang, 1973). They will alter the timing and duration of activity to avoid hyperthermia (MacArthur and Wang, 1974; Simpson, 2009). During the winter, pikas are dependent on the snowpack for insulation (Smith *et al.*, 2004). Temperatures in talus insulated by snow cover stay around 0°C, and loss or extreme reduction of that snow cover results in drastically variable within-talus

temperatures (Beever *et al.*, 2010). Both lack of snowpack and aberrant snowmelt have been linked to pika population decline and extirpation (Beever *et al.*, 2010; Morrison & Hik, 2007). Temperature sensitivity combined with high philopatry, small populations, and restriction to patchily distributed alpine habitat appears to make North American pikas especially vulnerable to extirpation due to climate change (Lawlor, 1998; Beever *et al.*, 2003; Krajick, 2004).

Support for Bergmann's rule in pikas has been mixed in previous studies. The Asian species *O. curzoniae* (the black-lipped pika; Lin *et al.*, 2008) conforms to Bergmann's rule, but an inverse Bergmannian relationship has been reported in the Asian *O. dauurica* (the Daurian pika; Liao *et al.*, 2007). As part of a meta-analysis, Ashton *et al.* (2000) reanalyzed average mass for populations of the American pika (*O. princeps*) originally published in Smith (1978). They reported a significant negative relationship between July temperatures and body size (as predicted by Bergmann's rule, but see reanalysis in Appendix C). However, other studies of *O. princeps* provide inconclusive support, reporting either larger skulls in northern populations (Bergmannian; Galbreath *et al.*, 2010), or a negative relationship with latitude (inverse-Bergmannian; Wisely, 1973). These differences in body size clines within pikas, long considered to be a fairly morphologically uniform genus (Corbet, 1978), are themselves of interest. My goal is to determine the presence and direction of latitudinal variation of body size within *O. collaris*, and if there are shifts in body size over the last century in response to climatic warming.

2.2.2 Hypotheses

This study has two objectives: first, to test for latitudinal and temporal variation within *O. collaris*; and second, to examine relationships between climate variables and body size. Two contrasting but not mutually exclusive hypotheses might account for clines in body size of small mammals:

H1: Resource availability (Figure 1a)– Warmer summers will lead to longer growing seasons and more primary productivity (Rosenzweig, 1968), resulting in a positive relationship between body size and temperature (Figure 1c);

Prediction 1a: Body size will increase over time due to increases in temperature.

Prediction 1b: Body size will decrease with increasing latitude.

H2: Thermoregulation (Figure 1b)– Given that smaller body sizes dissipate heat faster (Brown & Lee, 1969; James, 1970), warmer temperatures might favor smaller pikas, or, conversely, colder temperature might favor larger pikas (lower surface:volume ratio), resulting in a negative relationship between body size and temperature (Figure 1d):

Prediction 2a: Body size will decrease over time.

Prediction 2b: Body size will increase with latitude.

As arctic and subarctic temperatures have greatly increased over the last century (AICA, 2008), we would expect increasing size under the resource availability hypothesis and decreasing size under the thermoregulation hypothesis. Measuring the adaptive response over the short-term is important to understanding the degree to which ecotypic variation portends the effects of climate change (e.g., Berteaux *et al.*, 2004). Very few studies have focused on body size chronoclines in Alaskan mammals, and research on temporal changes of body size in the collared pika is particularly interesting given the temperature sensitivity of pikas and the rapid rate of climate change at high latitudes.

2.3 Methods

2.3.1 Data collection

Studies have suggested that choice of body size metric can affect the degree of fit to predicted patterns of ecogeographic variation (Meiri & Dayan, 2003). At the time of collection, five standard measurements are generally taken on nonvolant

terrestrial mammals: total length, tail length, hind foot length, ear from notch, and mass (g). These metrics provide a general indication of body size but are likely prone to greater measurement error than measurements recorded in a standardized fashion by a single investigator. To reduce measurement error, I contrasted measurements of total length and mass with those taken on a series of skulls from 223 museum specimens representing 110 years of samples from throughout the known distribution of the species (Figure 2; Appendix A). Greatest skull length (GSL; anterior-most point of the nasal bones to posterior-most margin of the supraoccipital taken in the dorsal aspect) was measured, as a proxy for overall body size, on each adult skull using digital calipers, with an accuracy of .01 mm. The relationship between specimen mass and GSL was verified with a Pearson correlation coefficient (n=122; r = 0.7748; Figure 3). Subadults were excluded from final analyses. Specimens were aged and excluded based on a combination of markers (not all data were available for each specimen): cranial suture ossification (as described in Weston, 1979), dental arcade incompletely erupted, unfused epiphyseal plates, grey juvenile pelage (no presence of molt), and mass at time of collection (< 100 g). Where available, collector-recorded variables were recorded from skin tags (sex, month of collection, year of collection, latitude, longitude, and elevation). When information on latitude, longitude, and elevation was not available from the institution, they were determined using the Berkeley georeferencing guidelines and BioGeomancer (http://bg.berkeley.edu/latest/). Localities with large inferred error (>22.5 km) were excluded from the analysis.

A series of possible causal variables (Table 1) was used to test for geographic and temporal variation, and explicitly address hypothesized changes in body size (Figure 1). First, I analyzed geographic and temporal models related to when and where a specimen was collected (collection model). Second, I included a series of variables intended to capture broad-scale global (Northern Hemisphere) climate patterns. These model comparisons included data on temperature anomalies and

the Pacific Decadal Oscillation (PDO). Northern Hemisphere temperature anomalies, computed relative to the period from 1951-1980, provide a broad-scale measure of temperature shifts, correlated over large regions (Hansen and Lebedieff, 1987; data downloaded from http://data.giss.nasa.gov/gistemp). The PDO index is the first principal component of a multivariate measure that reflects climate cycling with mean temperature anomalies removed in order to avoid capturing global warming signal (Mantua *et al.*, 1997; http://jisao.washington.edu/pdo/PDO.latest). During the positive phase of the PDO cycle, Alaska averaged 3.1ºC warmer than the negative phase, with increased precipitation and cloud cover (Hartmann and Wendler, 2005). The PDO index is strongly correlated with date of spring snowmelt for pika populations in the Yukon Territory, and the one-year lagged winter PDO values were positively correlated with adult pika survival (Morrison & Hik, 2007). In the third set of models, I included local temperature variables with previously discussed variables to compare a series of "local" models. I used temperatures (deviations in °C from the 1951-1980 average) from the Fairbanks, Alaska area (Alaska Climate Research Center [ACRC] data available at http://climate.gi.alaska.edu/Climate/index.html) for winter (December, January and February average; DJF), summer (June, July, and August average; JJA), and annual averages. Temperatures at the Fairbanks weather station were highly correlated (n=79, ρ_{avg}=0.70-0.88) with temperature at other weather stations in pika habitat in Alaska (Big Delta, Gulkana, Talkeetna, and McGrath; [ACRC data]). The temperature record at Fairbanks station extends back to 1930. Multiple regression models comparing local and global variables excluded specimens collected before 1930, limiting the temporal scope of the analyses ($n_{included}$ = 154).

2.3.2 Analyses

I used multiple linear regression models to examine latitudinal, temporal, and climate-related variation in body size. To broadly examine the relationship between

latitude and body size, greatest skull length (GSL), mass, and total length were each regressed against latitude. To decrease the potential sources of error, subsequent model fitting and multiple regressions were conducted on GSL only. For all models, relationships between explanatory variables were examined using a correlation matrix. Collinearity was avoided through the examination of tolerance values and the exclusion of variable combinations that were highly collinear; tolerance values of approach 1 when variables are uncorrelated, but only tolerances of 0.1 or lower will inflate the standard error of the regression coefficient (Quinn & Keough, 2002). To select the best model within each set I used the Akaike Information Criterion (AIC). This approach protects against overfitting by weighing explained variance against model complexity (Burnham & Anderson 2002). I used the AIC correction for small sample size (AIC_c; Burnham & Anderson, 2002), cases where $n/K < 40$. Values for ΔAIC_c (the difference between AIC for a particular model and the lowest AIC score) and AIC weights were calculated for each model. Both parameters compare the difference between models, with ΔAIC_c scores that range from 0-2 being equivalent to essentially identical support (Burnham & Anderson, 2001). As the goal of this study involves determining important variables and not estimating parameters, models and interaction terms were compared using stepwise variable selection. Stepwise methods have been criticized for leading to bias in parameter estimates and being overly focused on choosing the single best model (Wittingham *et al.* 2006). However, all subsets approaches, which are generally considered to be better for prediction, can be susceptible to problems involving collinearity between predictor variables (Quinn & Keough, 2002). To address any problems arising from the uneven distribution of historically collected samples, all regression models were compared with and without collecting locality included as a random effects variable. Residual and normal probability plots were used to check the fit of the model to the underlying assumptions. All analyses were run in the R statistics package (v. 2.9.2 http://www.r-project.org/).

As a complement to multiple regression models, I employed a conditional regression tree approach to characterize the relationship between GSL and the explanatory variables (Table 1; De'ath & Fabricius, 2000). This approach explains variation in the data by recursively partitioning it into binary explanatory groupings and may reveal patterns that regression models fail to find (De'ath & Fabricius, 2000; Quinn & Keough, 2002).

Support for geographic and/or temporal trends in GSL was determined through presence of a significantly non-zero slope (regression coefficient) with regard to latitude and year. The two hypotheses regarding process were evaluated based on the sign of the slope and its relationship to temperature and year of collection. The resource availability hypothesis predicts a positive relationship between GSL and temperature, resulting in a positive relationship with year and a negative relationship with latitude. In contrast, the thermoregulation hypothesis predicts a negative relationship between GSL and temperature that would result in a negative relationship with year and a positive relationship with latitude. To further compare these hypotheses, I compared body size metrics from pikas collected in 1962 near Paxson, AK with those from a 2007 resurvey of the same locality. The 1962 series represents the largest historically sampled population of collared pikas with locality information of a sufficient quality to permit resampling. In addition to mass, total length, and greatest skull length, I used digital calipers to measure eight additional cranial variables intended to capture overall changes in size (museum catalog numbers, measurements, and collection information given in Appendix B). Population means were compared with Welch's two-sample t-test, corrected for unequal variance (Quinn & Keough, 2002).

2.4 Results

The best-supported pattern was an inverse relationship between GSL and latitude, which was supported by the relationship between mass and latitude

(Figure 4). Total length was not significantly related to latitude. These findings were further supported by a conditional regression tree approach, which indicated that the majority of the discontinuities in GSL are explained primarily by latitude and secondarily by sex (Figure 5). Collared pikas exhibited a slight sexual size dimorphism, with males slightly larger than females (Figure 5). Although the categorical analysis and regression tree approach indicated that sexual size dimorphism might be more pronounced at latitudes below 62.4°N, the sex-by-latitude interaction term was not significant ($p_{var} = 0.479$).

Model selection using the AIC_c criterion further indicated that both latitude and sex belong in the best subset of models (Table 2). Removing latitude from the best collection model resulted in ΔAIC_c of 67.37. The best subset of collection models included latitude, sex, and year of collection. Adding longitude or date to those models resulted in essentially identical support based on $\Delta AIC_c < 2$. Variables in the best single collection model (Table 3) and the other highly ranked models (Table 2) supported the same relationship between GSL and a given predictor variable (e.g., positive or negative sign for a given partial regression coefficient). The best global climate model included latitude, sex, and midwinter temperature anomalies in the Northern Hemisphere (ano.DJF). Out of the set of nine equally supported global models (best 5 shown in Table 2), latitude, sex, and ano.DJF variables were included in all but one (latitude, sex, and year). Other variables included in equivalently supported global models were not significant at the $\alpha = 0.05$ level. The inclusion of additional variables (mostly values for the PDO) in the global models did not change the sign of the partial regression coefficients for latitude, sex, or ano.DJF. Though none of the PDO index variables were significant, all of the highly supported models that included PDO values indicated a negative relationship between PDO and GSL. None of the models that included locality as a random effects variable were well supported based on AIC_c scores. Comparisons of models regressing local, global, and collecting variables against specimens collected

since 1930 resulted in three best models, all of which included latitude, sex, and summer Fairbanks temperature (faiT.JJA) variables. Equally supported models included additional non-significant explanatory variables that did not affect the sign of relationship between GSL and the partial correlation coefficients. All of the best models indicated a negative relationship between GSL and latitude. The best subsets of collection and global models both indicated a positive relationship between GSL and year of collection or winter temperature anomalies. The best subset of the local models indicated a negative relationship between GSL and local summer temperature but as these models compared a reduced temporal range of samples they may not be directly comparable with the longer dataset.

Although the dataset is small, trends from the resampled site were in agreement with the broader signal of increasing size over time (Figure 6). Although not all of the comparisons were statistically significant, six of the seven significant means for cranial metrics showed increases over time (Table 4). Population means for total body length were significantly less in 2007 than 1962.

2.5 Discussion

Body size (GSL and mass) in the collared pika is negatively correlated with latitude, and therefore inversely Bergmannian. Inverse Bergmannian trends are often explained by empirical relationships between body size and food availability or inferred by contrasting body size and climate change (Meiri *et al.*, 2007, Yom-Tov & Yom-Tov, 2005). Small mammals such as the vole genus *Microtus* (Ashton *et al.*, 2000), the shrew genus *Sorex* (Ochocinska & Taylor, 2003; Yom-Tov & Yom-Tov, 2005), and at least one species of tenrec (*Microgale majori*, Olson *et al.*, 2004) show inverse Bergmannian patterns. Burrowing mammals have long been considered to be exceptions to Bergmann's rule (Mayr, 1963) as they are insulated from temperature fluctuations by both soil and snowpack. While not strictly burrowing (Smith *et al.*, 1990), Nearctic pikas are thought to rely heavily on the snowpack for

overwinter insulation (Beever *et al.*, 2010; Morrison *et al.*, 2007). The latitudinal and temporal trends from our analyses support the hypothesis that trends in primary productivity instead of thermoregulation pressure are influencing body size along a latitudinal gradient. Trends in precipitation could also play a role in body size clines in collared pikas (e.g., Yom-Tov & Geffen, 2006; Blois *et al.*, 2008); however, a fine-scale analysis would be needed to address those effects.

North American pikas are facultatively monogamous, maintain territories and food caches as individuals rather than mated pairs, and are distributed in an alternating male-female pattern on the landscape. These traits might be expected to lead to little or no sexual dimorphism in size. Indeed, no sexual dimorphism has been found in either the American pika, sister to the collared pika (Wisely, 1973; Weston, 1979) or the northern pika (*O. hyperborea*; Lissovsky, 2003) from Asia. While the CART analyses indicated an effect of sex at lower latitudes, the interaction of sex and latitude was not significant in the multiple regression models, nor were these models within the best set based on AIC_c scores. The support for this pattern is equivocal. Variation in the degree of dimorphism by latitude (Rensch's rule; Fairbairn, 1997; Blankenhorn *et al.*, 2006) could be indicative of localized ecological or behavioral differences (e.g., Storz *et al.*, 2001). Although dimorphism in collared pikas is not as strong as that observed in some other mammalian groups (e.g., carnivorans), it is clearly an important factor to test and account for in future morphological analyses within Ochotonidae.

Pacific Decadal Oscillation index variables (average at different annual and temporal scales) were among the subset of nine equivalent global models, but were never statistically significant components of the multiple regression models. This index has been found to negatively correlate with the date of snowmelt and positively correlate with overwinter survivorship in collared pikas in the southwest Yukon Territory (Morrison & Hik, 2007). Based on latitudinal trends, snowmelt date might be expected to affect pika body (and skull) size positively through

increasing the length of the growing season, although partial correlation coefficients for PDO variables in all models were negative. Values for the PDO index may not be strongly related to body size changes over time or they may be more apparent in a fine-scale analyses. Likewise, responses to precipitation are probably important, but precipitation can be locally variable. Better models of historical changes in local precipitation and snowpack are needed to fully address these relationships (e.g., Blois *et al.*, 2008).

The most surprising results from this study involved the relationships between GSL, year and climate variables (Tables 1 and 2). The negative relationship between latitude and body size led to the prediction that year would be positively correlated with body size (which it was). Likewise, winter temperature anomalies (ano.DJF) from the best global models were also positively correlated with body size. These results support the resource availability hypothesis, but this hypothesis seems to be contradicted by the best local model (which was also the best overall model). The subset of best local models reflect a negative relationship between GSL and local summer temperatures (faiT.JJA). Hence, collared pikas appear simultaneously to be getting smaller during years with warm summers and larger during years with high winter temperature anomalies. While this discrepancy might result from differences in the temporal range of data used for the within-subset model comparisons, it might also be explained by thermoregulatory selection pressure. Warmer summers in interior Alaska are strongly correlated (ρ_{avg}=0.70) with warmer summers in collared pika habitat. These warmer summers may select for smaller pikas, which would be better at shedding heat and maintaining haying activity during summer hours (Morrison *et al.*, 2009; MacArthur & Wang, 1974). Results from the best global models (comparing the full range of years) could also be interpreted in the context of thermoregulation. Northern Hemisphere winter temperature anomalies (ano.DJF) are not tightly correlated with local summer temperatures (faiT.JJA; ρ=0.21), but are related to midwinter thaws and aberrant

melt events (Karl *et al.*, 1993). Midwinter snowmelt and rain destroy protective snow cover and can be devastating on pika populations (Smith *et al.*, 2004; Morrison & Hik 2007). Midwinter thaws were associated with a catastrophic 90% population decline at one study site in the southwest Yukon Territory (Morrison & Hik 2008). These aberrant midwinter events may produce strong selective sweeps that eliminate smaller pikas with larger surface:volume ratios. Under this scenario, body size will increase as the frequency of midwinter melting events increases, as anticipated under global warming models (IPCC, 2001; ACIA, 2008; Post *et al.*, 2009). Support for this hypothesis may be found in the resurvey site comparisons that suggest that pikas have become larger overall but shorter in total length. Cranial measurements that co-vary with overall pika body mass show an increase over the past half-century, while total length showed a significant decrease (Table 4, Figure 6). This should result in a smaller surface:volume ratio. Although based on a seemingly small sample from a limited timescale, these results are consistent with the broader temporal trends evident in the larger dataset. While larger body size overall might be explained by increases in resource availability, greater mass but shorter total length seems unlikely under the resource availability hypothesis.

> *"Continued collecting efforts should perhaps be more appropriately viewed as an obligation if we are to continue to rigorously and responsibly document the changes we are causing."*
>
> Millien *et al.* 2006, p. 862

Quantifying the complex interplay between climate change and species responses requires concerted monitoring and prediction efforts. The unprecedented rate of recent warming indicates the severity of this threat, as it outpaces any other major climatic transition mammals have experienced (Blois & Hadly, 2009). For the Arctic in particular, the inclusion of historically collected specimens can extend the range of inference back over the previous century. Arctic warming has been 2-3

times the rate of global warming, and changes in temperature, snow, ice cover, and nutrients are causing severe perturbations (Post *et al.*, 2009). In addition to the disastrous effects of mid-winter warming on the insulating snowpack (Smith *et al.*, 2004, Morrison & Hik, 2007), Arctic winter warming can also damage plant productivity (Post *et al.*, 2009), further threatening herbivorous pikas. While the continuation of the International Polar Year studies provides critical monitoring, museum specimens can and should complement this effort, providing "canaries in the coal mine" for the monitoring of anthropogenic climate change (Graham *et al.*, 2004; Winker, 2004).

2.6 Acknowledgements

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2.7 Figures

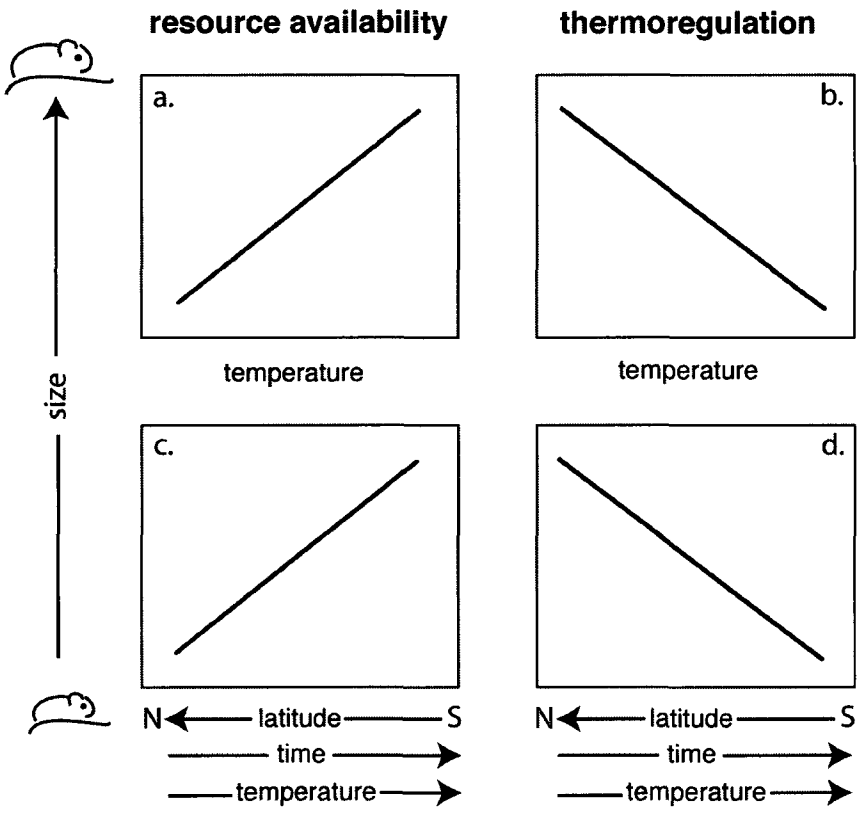

Figure 1 Relationship between body size and under the resource availability and thermoregulation hypotheses. Clinal variation in body size of collared pikas may be explained by gradients in resource availability, under which body size is positively correlated to temperature (a), or by thermoregulation pressures, which would lead to a negative correlation between body size and temperature (b). The thermoregulation hypothesis predicts body size will increase at lower latitudes and over time due to global warming (c). Under the thermoregulation hypothesis (d), body size will increase at higher latitudes (Bergmann's rule) and decrease over time.

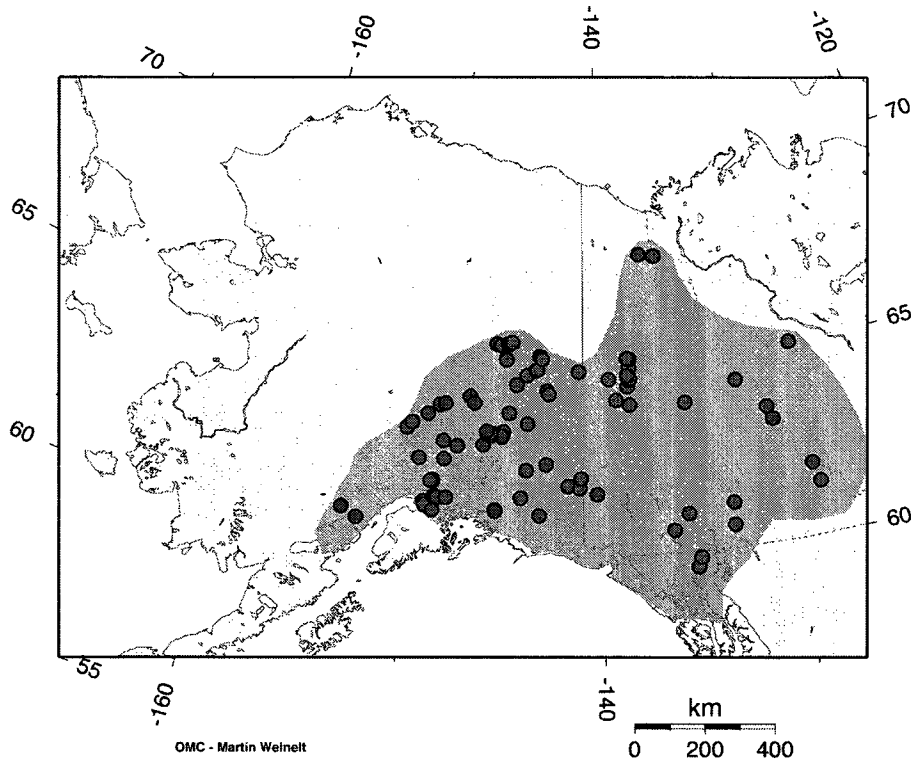

Figure 2 Distribution of *Ochotona collaris* (dark grey, modified from the International Union for the Conservation of Nature; www.iucnredlist.org) and sampling localities included in this study

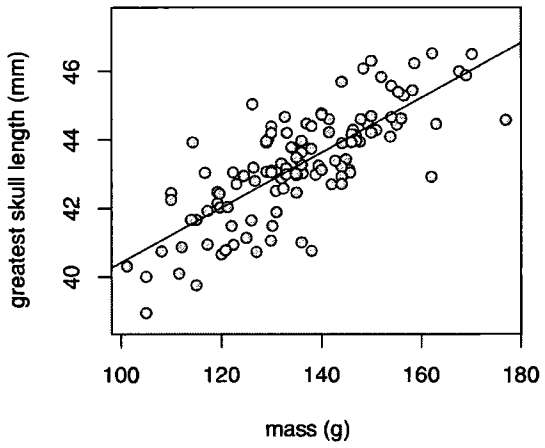

Figure 3 Correlation between greatest skull length and mass (n = 122, $R^2 = 0.597$, $P < 0.00001$)

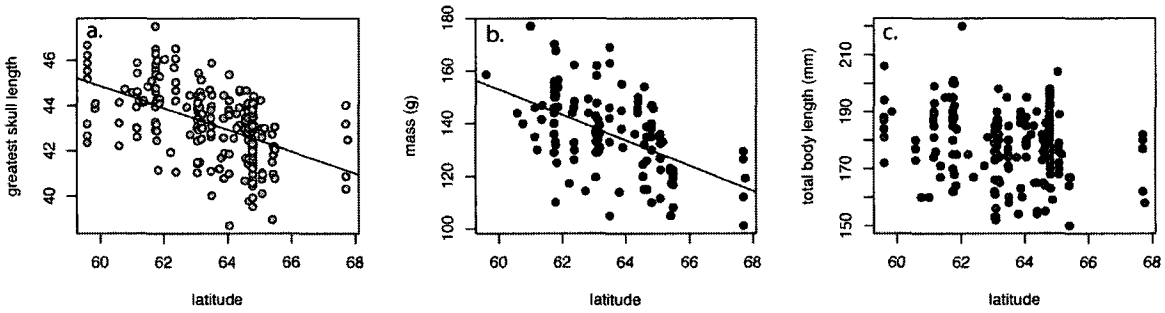

Figure 4 Relationship between (a) greatest skull length and latitude ($F_{1,215}$ = 77, adj. R^2 = 0.26, $p < 0.0001$), (b) mass and latitude ($F_{1,116}$ = 44.8, adj. R^2 = 0.27, $p < 0.0001$), and (c) total body length and latitude ($F_{1,194}$ = 3.26, adj. R^2 = 0.01, p = 0.07). Latitude is shown from south to north, not north to south as in Figure 1.

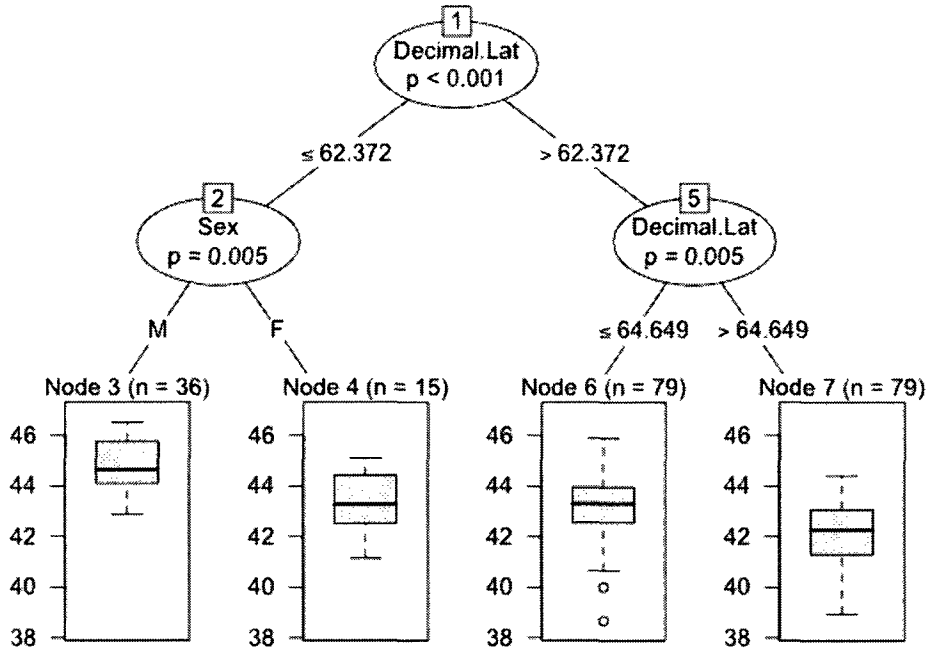

Figure 5 Conditional regression tree model of the relationship of skull length to decimal latitude and sex. Significance of each node is shown with criteria for cut-off shown along each branch. Boxplots at tips indicate distribution of skull length at terminal nodes (with n = the number of observations at a particular node).

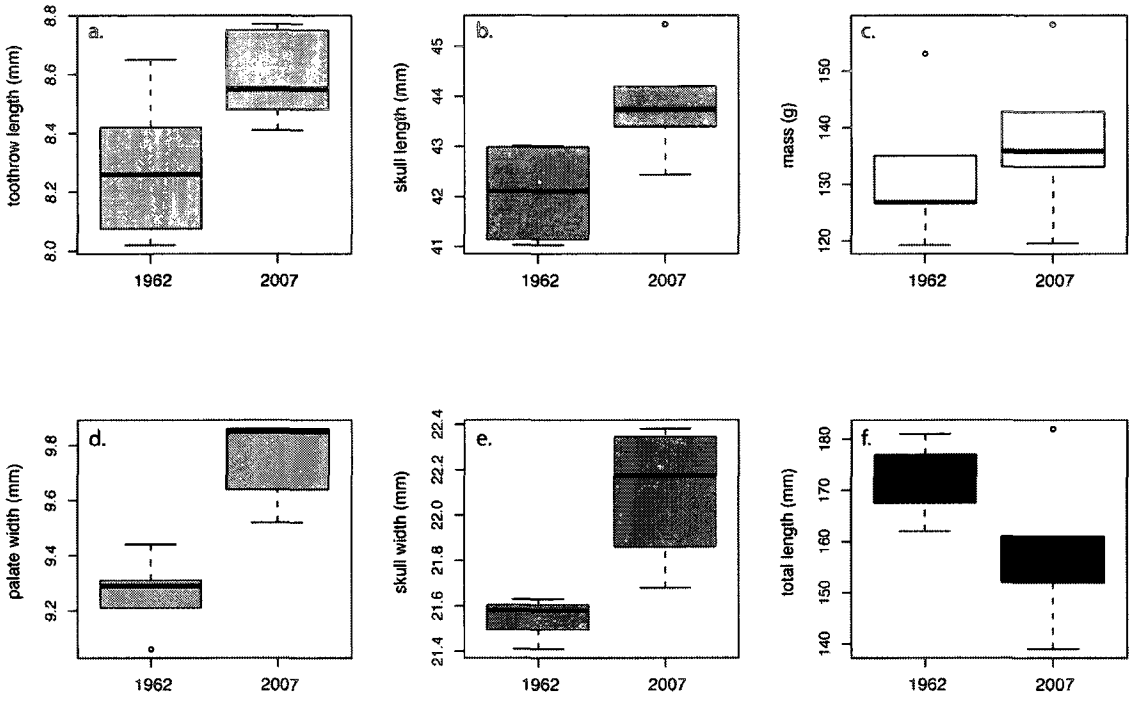

Figure 6 Change in several body size proxies for collared pikas in serially collected samples. Collared pikas show increases in (a) toothrow length, (b) greatest skull length, (c) mass (non-significant), (d) palate width at the second premolar, and (e) maximum skull width measured at the zygomatic arch. (f) Total length (as measured by collector) decreased significantly between time periods.

2.8 Tables

Table 1 – Variables included in multiple regression models. Each set of model comparisons included non-collinear combinations of variables from the proceeding set (i.e., global climate models included combinations of global and collection variables). Models compared under the local climate set were regressed against GSL for specimens collected since 1941. Variables with the .JJA suffix represent June, July, and August averages. Variables with the .DJF suffix represent an average for December, January, and February values. The suffix .ann refers to annual averages.

collection models	n = 217
latitude	latitude of collection
longitude	longitude of collection
year	year of collection
sex	sex of individual
date	standardized date (days +/- the average date of collection)
"global" climate models	n = 217
temperature anomalies	
ano.DJF	Northern Hemisphere winter temperature anomalies (fractions of degrees C +/- the 1951-1980 average)
ano.JJA	Northern Hemisphere summer temperature anomalies (fractions of degrees C +/- the 1951-1980 average)
Pacific decadal oscillation	
PDO.JJA	PDO index average over June, July, and August
PDO.DJF	PDO index average over December, January, and February
PDO.winter	PDO index average over winter months (December through April)
PDO.ann	annual PDO index
PDO.Wlag	PDO index over winter months lagged by 1 year
PDO.Slag	PDO index over summer months lagged by 1 year
"local" climate models	n = 154
local temperatures	
faiT.JJA	Fairbanks temperature average during June, July, and August (fractions of degrees C +/- the 1951-1980 average)
faiT.DJF	Fairbanks temperature average during December, January, and February (fractions of degrees C +/- the 1951-1980 average)
faiT.ann	Fairbanks annual average temperature (fractions of degrees C +/- the 1951-1980 average)

Table 2 – The best five multiple regression models based on the Akaike Information Criterion (AIC) corrected for small sample size (AIC_c). R^2 is the adjusted R^2 for the model, ΔAIC_c is the difference between AIC_c for each model and the best model, W_{AICc} is the Akaike weight.

	R^2	AIC_c	ΔAIC_c	W_{AICc}
collection models (n = 217)				
latitude + sex + year	0.326	738.8	0	0.43
latitude + sex + year + longitude	0.313	740.6	1.76	0.18
latitude + sex + year + date	0.314	740.6	1.78	0.17
latitude + sex	0.301	742.2	3.43	0.08
latitude + sex + year + date + longitude	0.311	742.2	3.47	0.08
global climate models (n = 217)				
latitude + sex + ano.DJF	0.321	737.3	0	0.16
latitude + sex + ano.DJF + PDO.winter	0.321	738.1	0.79	0.11
latitude + sex + ano.DJF + PDO.JJA	0.321	738.3	0.95	0.10
latitude + sex + ano.DJF + PDO.Wlag	0.320	738.4	1.13	0.09
latitude + sex + ano.DJF + PDO.ann	0.320	738.5	1.21	0.09
local climatic models (n = 154)				
latitude + sex + faiT.JJA	0.338	525.6	0	0.23
latitude + sex + year + faiT.JJA	0.337	527.0	1.46	0.11
latitude + sex + faiT.JJA + ano.JJA	0.334	527.2	1.67	0.10
latitude + sex + faiT.JJA + PDO.Wlag	0.336	527.7	2.14	0.08
latitude + sex + year + faiT.JJA + ano.DJF	0.336	527.7	2.16	0.08

Table 3 – Summary of variables in the best multiple regression models (based on AIC_c) under each set of model comparisons. Partial regression coefficients, standard error (SE), tolerance (tol), and the significance (p_{var}) are given for each variable. Adjusted R^2 and significance (p_{model}) are given for the model as a whole.

Model	coefficient	SE	tol	p_{var}	R^2	p_{model}
collection model					0.326	***
intercept	58.33	6.60	-	***		
latitude	-0.45	0.05	0.98	***		
sex	0.70	0.18	0.99	***		
year	0.007	0.002	0.98	*		
global model					0.321	***
intercept	70.50	3.36	-	***		
latitude	-0.44	0.05	0.98	***		
sex	0.69	0.18	0.99	**		
ano.DJF	0.005	0.002	0.98	**		
local model					0.338	***
intercept	74.54	4.18	-	***		
latitude	-0.36	0.06	0.91	***		
sex	0.77	0.21	0.99	**		
faiT.JJA	-0.145	0.06	0.92	*		

Significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4 – Comparison of body size metrics from pikas sampled in 1962 and 2007 at the resampled site. Number of individuals from each sampling period, population averages, t-statistic, p-value, and sign of interaction are given. Measurement descriptions are given in Appendix B.

metric	n_{1962}	n_{2007}	$mean_{1962}$	$mean_{2007}$	t	p-value	sign
mass (g)	5	6	132.14	137.56	-0.697	0.504	+
total length (mm)	7	6	172.57	156.67	**2.470**	**0.042**	-
toothrow length (mm)	7	5	8.27	8.59	**-2.730**	**0.021**	+
infraorbital breadth (mm)	7	6	6.11	6.41	**-2.495**	**0.035**	+
orbital breadth (mm)	6	6	5.49	5.60	-0.764	0.463	+
greatest skull length (mm)	4	5	42.07	43.84	**-2.434**	**0.047**	+
skull breadth (mm)	3	4	21.54	22.10	**-3.267**	**0.031**	+
breadth at notch (mm)	5	5	5.35	5.46	-0.949	0.378	+
palate width (at P2; mm)	5	5	9.26	9.75	**-5.139**	**0.001**	+
palate width (at P4; mm)	5	5	12.00	12.33	**-2.449**	**0.041**	+
palate width (at M2; mm)	5	5	12.65	12.64	0.072	0.946	-

2.9 References

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2.10 Appendices

Appendix A – Specimens examined. CMN= Canadian Museum of Nature, KU = University of Kansas Natural History Museum & Biodiversity Research Center, MSB = Museum of Southwestern Biology, UAM = University of Alaska Museum, USNM = US National Museum

Museum	Cat. No.	State/Province	Decimal Lat	Decimal Long	Year	GSL (mm)	Sex
CMN	5634	Alaska	61.497221	-144.4194412	1925	44.83	M
CMN	17328	Yukon Territory	61.6168432	-140.2624512	1943	45.10	F
CMN	17802	Yukon Territory	61.166667	-132.983333	1944	42.60	F
CMN	17803	Yukon Territory	61.166667	-132.983333	1944	45.43	M
CMN	17824	Yukon Territory	61.166667	-132.983333	1944	44.61	M
CMN	17828	Yukon Territory	61.166667	-132.983333	1944	45.89	M
CMN	17829	Yukon Territory	61.166667	-132.983333	1944	44.56	F
CMN	17831	Yukon Territory	61.166667	-132.983333	1944	42.99	F
CMN	18106	Northwest Territories	65.0350604	-128.4631348	1944	44.22	M
CMN	18120	Yukon Territory	63.1666666	-130.2	1944	45.14	M
CMN	29410	Yukon Territory	64.6485844	-138.3425903	1961	44.10	F
CMN	29411	Yukon Territory	64.6485844	-138.3425903	1961	43.84	F
CMN	29413	Yukon Territory	64.6485844	-138.3425903	1961	43.44	M
CMN	29414	Yukon Territory	64.6485844	-138.3425903	1961	42.82	M
CMN	29415	Yukon Territory	64.6485844	-138.3425903	1961	42.75	F
CMN	29416	Yukon Territory	64.6485844	-138.3425903	1961	41.41	F
CMN	30302	Northwest Territories	67.7084535	-136.2340937	1962	43.19	M
CMN	30303	Northwest Territories	67.7084535	-136.2340937	1962	40.31	F
CMN	30304	Northwest Territories	67.7084535	-136.2340937	1962	44.02	M
CMN	30647	Yukon Territory	64.5833282	-139.4999924	1962	43.45	M
CMN	30648	Yukon Territory	60.5950594	-133.0718994	1962	44.13	F
CMN	30649	Yukon Territory	60.5950594	-133.0718994	1962	42.21	F
CMN	30650	Alaska	63.1875038	-145.5580597	1962	42.51	M
CMN	30651	Alaska	63.1875038	-145.5580597	1962	43.25	F
CMN	30652	Alaska	63.1875038	-145.5580597	1962	43.02	F
CMN	30653	Alaska	63.06937	-146.21866	1962	44.47	M
CMN	30654	Alaska	63.06937	-146.21866	1962	43.88	M

CMN	30655	Alaska	63.06937	-146.21866	1962	43.07	M
CMN	31161	Yukon Territory	61.0010814	-135.4147338	1963	44.58	F
CMN	31163	Yukon Territory	61.8716911	-128.5180664	1963	44.29	F
CMN	31164	Yukon Territory	61.8716911	-128.5180664	1963	44.09	M
CMN	31165	Yukon Territory	61.8716911	-128.5180664	1963	45.29	M
CMN	31166	Yukon Territory	61.3651452	-128.2653809	1963	44.13	M
CMN	31167	Yukon Territory	61.3651452	-128.2653809	1963	44.22	M
CMN	31170	Yukon Territory	64.3850663	-138.3782959	1963	43.12	F
CMN	31171	Yukon Territory	64.3850663	-138.3782959	1963	43.90	M
CMN	31172	Yukon Territory	64.3850663	-138.3782959	1963	44.60	M
CMN	31174	Yukon Territory	64.3850663	-138.3782959	1963	44.69	M
CMN	31175	Yukon Territory	64.87478	-138.25229	1963	43.04	F
CMN	31201	Yukon Territory	63.87939	-135.0837707	1963	44.44	M
CMN	31202	Yukon Territory	63.87939	-135.0837707	1963	40.76	F
CMN	31203	Yukon Territory	63.87939	-135.0837707	1963	43.43	F
CMN	33697	Yukon Territory	67.7677132	-137.2192382	1964	42.48	F
CMN	35314	Yukon Territory	64.2999992	-131.9999924	1966	43.63	M
CMN	35316	Yukon Territory	63.4999962	-130.4333344	1966	43.10	F
CMN	35318	Yukon Territory	63.4999962	-130.4333344	1966	42.70	F
CMN	35321	Yukon Territory	63.4999962	-130.4333344	1966	43.97	F
CMN	35324	Yukon Territory	63.4999962	-130.4333344	1966	40.01	F
CMN	35329	Yukon Territory	63.4999962	-130.4333344	1966	45.88	F
CMN	35330	Yukon Territory	63.4999962	-130.4333344	1966	44.46	F
CMN	35331	Yukon Territory	63.916667	-138.3	1966	41.89	F
CMN	40302	Alaska	63.166667	-146.5	1969	44.39	M
CMN	40303	Alaska	63.166667	-146.5	1969	43.10	M
CMN	40304	Alaska	63.133333	-146.25	1969	44.60	M
CMN	40305	Alaska	63.133333	-146.25	1969	43.92	M
CMN	40306	Alaska	63.133333	-146.25	1969	41.49	F
CMN	40307	Alaska	63.133333	-146.25	1969	43.78	F
CMN	40308	Alaska	61.783333	-149.333333	1969	43.17	M
CMN	40309	Alaska	61.783333	-149.333333	1969	44.28	M
CMN	40310	Alaska	61.783333	-149.333333	1969	44.21	M
CMN	40311	Alaska	61.7474562	-149.2341613	1969	43.27	F
CMN	40312	Alaska	61.7474562	-149.2341613	1969	42.93	F
CMN	40313	Alaska	61.7474562	-149.2341613	1969	43.96	M
CMN	40314	Alaska	61.7474562	-149.2341613	1969	44.16	M

CMN	40315	Alaska	61.7474562	-149.2341613	1969	43.97	M
CMN	40316	Alaska	61.7474562	-149.2341613	1969	44.77	M
CMN	40317	Alaska	61.7474562	-149.2341613	1969	45.57	M
CMN	40318	Alaska	61.7474562	-149.2341613	1969	44.62	M
CMN	44998	Yukon Territory	65.1	-138.366667	1977	40.94	F
CMN	45000	Yukon Territory	65.083333	-138.266667	1977	40.73	F
CMN	45002	Yukon Territory	65.083333	-138.216667	1977	41.01	F
CMN	45003	Yukon Territory	65.083333	-138.216667	1977	42.58	M
CMN	45005	Yukon Territory	65.083333	-138.216667	1977	40.10	M
CMN	45006	Yukon Territory	65.083333	-138.216667	1977	42.80	M
CMN	45007	Yukon Territory	64.666667	-138.383333	1977	42.46	F
CMN	45009	Yukon Territory	64.666667	-138.383333	1977	42.98	F
CMN	45012	Yukon Territory	64.55	-138.25	1977	42.95	F
CMN	45013	Yukon Territory	64.55	-138.25	1977	40.66	F
CMN	46541	Northwest Territories	67.7084535	-136.2340937	1973	40.87	F
CMN	50386	Alaska	63.0899982	-146.4350128	1981	43.94	M
CMN	50387	Alaska	63.0899982	-146.4350128	1981	42.92	F
CMN	50388	Alaska	61.7474562	-149.2341613	1981	46.50	M
CMN	50389	Alaska	61.7474562	-149.2341613	1981	45.39	M
CMN	50390	Alaska	61.7474562	-149.2341613	1981	45.83	M
CMN	50391	Alaska	61.7474562	-149.2341613	1981	46.30	M
KU	29090	British Columbia	59.599	-136.422	1948	42.35	F
KU	29092	British Columbia	59.599	-136.422	1948	45.89	M
KU	29093	British Columbia	59.599	-136.422	1948	46.67	F
KU	29094	British Columbia	59.599	-136.422	1948	45.53	F
KU	29096	British Columbia	59.599	-136.422	1948	45.19	F
KU	29097	British Columbia	59.599	-136.422	1948	42.66	M
KU	29099	British Columbia	59.599	-136.422	1948	43.18	M
KU	147382	Yukon Territory	61.743	-140.174	1951	47.51	M
MSB	85661	Alaska	62.3722878	-148.7316589	1956	46.52	M
MSB	85662	Alaska	62.3722878	-148.7316589	1950	44.21	F
MSB	85663	Alaska	61.7932513	-149.2101287	1957	42.87	M
MSB	85664	Alaska	61.7932513	-149.2101287	1955	46.00	M
MSB	85666	Alaska	62.3238907	-150.109436	1958	45.69	M
MSB	85667	Alaska	62.3722878	-148.7316589	1955	46.08	M
MSB	85668	Yukon Territory	64.5833282	-139.4999924	1964	44.05	NA
MSB	85673	Yukon Territory	64.5833282	-139.4999924	1964	43.31	F

MSB	85674	Alaska	62.8162735	-146.6400146	1955	44.45	F
MSB	136557	Alaska	62.3722878	-148.7316589	1954	44.67	M
MSB	136558	Alaska	62.3722878	-148.7316589	1954	45.04	F
MSB	137016	Alaska	62.3865505	-143.0667114	1961	41.06	M
MSB	137545	Alaska	62.2218763	-144.1571044	1965	41.93	F
UAM	1907	Alaska	64.66666667	-144.3333333	1956	42.85	NA
UAM	1921	Alaska	64.66666667	-144.3333333	1956	43.73	M
UAM	1924	Alaska	64.66666667	-144.3333333	1956	39.76	M
UAM	2785	Alaska	64.66666667	-144.3333333	1956	41.65	M
UAM	2950	Alaska	65.4	-145.9833333	1957	38.95	F
UAM	7085	Alaska	65.43333333	-145.5333333	1962	40.95	F
UAM	7089	Alaska	63.05	-145.6	1962	43.01	M
UAM	7090	Alaska	63.05	-145.6	1962	41.03	F
UAM	7095	Alaska	65.06666667	-145.65	1962	42.97	M
UAM	7107	Alaska	65.05	-145.6166667	1962	41.25	NA
UAM	13493	Alaska	62.73333333	-148.05	1980	43.93	M
UAM	13596	Alaska	62.8365279	-148.8704071	1980	42.71	F
UAM	15740	Yukon Territory	64.58333333	-138.2166667	1984	41.67	F
UAM	15743	Yukon Territory	64.58333333	-138.2166667	1984	44.66	M
UAM	15746	Alaska	65.4	-145.9666667	1984	41.49	F
UAM	15747	Alaska	65.4	-145.9666667	1984	42.71	M
UAM	31647	Alaska	61.21666667	-149.5833333	1994	44.20	M
UAM	35126	Alaska	59.6	-135.1666667	1995	46.23	M
UAM	56067	Alaska	61.78635	-141.20945	2001	42.45	F
UAM	56817	Alaska	61.83333333	-141.8333333	2001	41.14	F
UAM	57920	Alaska	62.03118333	-141.1294833	2001	46.05	M
UAM	58216	Alaska	64.8158	-143.7469	2001	43.05	M
UAM	58219	Alaska	64.8158	-143.7469	2001	43.12	F
UAM	58242	Alaska	64.811	-143.779	2001	44.40	F
UAM	58245	Alaska	64.811	-143.779	2001	43.97	M
UAM	58250	Alaska	64.8144	-143.7399	2001	42.25	M
UAM	58251	Alaska	64.8144	-143.7399	2001	43.48	M
UAM	63931	Alaska	65.48333333	-145.4166667	1995	42.03	M
UAM	63933	Alaska	65.48333333	-145.4166667	1995	42.04	M
UAM	63934	Alaska	65.48333333	-145.4166667	1995	40.78	F
UAM	63935	Alaska	65.48333333	-145.4166667	1995	42.15	M
UAM	63936	Alaska	65.48333333	-145.4166667	1995	43.04	F

UAM	63937	Alaska	65.48333333	-145.4166667	1995	43.05	F
UAM	63938	Alaska	65.48333333	-145.4166667	1995	40.75	M
UAM	71652	Yukon Territory	60.59805556	-136.255	1999	43.22	M
UAM	93191	Alaska	60.76666667	-153.85	2003	44.73	M
UAM	98296	Alaska	63.80186111	-148.981	2005	41.67	M
UAM	100896	Alaska	61.153	-145.7507778	2007	43.93	M
UAM	100898	Alaska	61.14188889	-145.7570278	2007	43.01	M
UAM	102507	Alaska	63.085542	-145.630703	2007	43.74	F
UAM	102495	Alaska	63.085083	-145.632944	2007	45.44	M
UAM	102496	Alaska	63.05764	-145.62511	2007	42.43	F
UAM	102497	Alaska	63.06709	-145.65366	2007	43.39	M
UAM	102487	Alaska	63.07098	-145.6382	2007	44.20	F
UAM	102554	Alaska	65.4405725	-146.2692261	2006	41.83	M
UAM	102555	Alaska	65.4405725	-146.2692261	2006	42.18	M
USNM	99192	Alaska	59.6122207	-135.1372895	1899	44.29	M
USNM	127142	British Columbia	59.8500023	-134.9833374	1903	44.08	M
USNM	128581	British Columbia	59.8500023	-134.9833374	1903	43.86	M
USNM	131258	Alaska	64.7852433	-141.2567139	1903	41.40	F
USNM	131261	Alaska	64.7852433	-141.2567139	1903	41.54	F
USNM	131264	Alaska	64.7852433	-141.2567139	1903	40.54	M
USNM	131266	Alaska	64.7852433	-141.2567139	1903	43.79	M
USNM	131268	Alaska	64.7852433	-141.2567139	1903	43.96	M
USNM	131269	Alaska	64.7852433	-141.2567139	1903	39.52	M
USNM	131270	Alaska	64.7852433	-141.2567139	1903	40.43	F
USNM	131275	Alaska	64.7852433	-141.2567139	1903	41.29	M
USNM	131276	Alaska	64.7852433	-141.2567139	1903	40.95	M
USNM	131277	Alaska	64.7852433	-141.2567139	1903	39.91	M
USNM	131280	Alaska	64.7852433	-141.2567139	1903	42.21	F
USNM	131285	Alaska	64.7852433	-141.2567139	1903	41.78	M
USNM	131287	Alaska	64.7852433	-141.2567139	1903	42.49	F
USNM	131288	Alaska	64.7852433	-141.2567139	1903	42.30	M
USNM	131292	Alaska	64.7852433	-141.2567139	1903	43.71	M
USNM	131293	Alaska	64.7852433	-141.2567139	1903	41.97	M
USNM	131294	Alaska	64.7852433	-141.2567139	1903	44.09	M
USNM	131296	Alaska	64.7852433	-141.2567139	1903	43.65	M
USNM	131297	Alaska	64.7852433	-141.2567139	1903	42.99	M
USNM	131298	Alaska	64.7852433	-141.2567139	1903	44.13	F

USNM	131301	Alaska	64.7852433	-141.2567139	1903	43.08	M
USNM	131303	Alaska	64.7852433	-141.2567139	1903	41.89	F
USNM	131304	Alaska	64.7852433	-141.2567139	1903	42.13	F
USNM	131309	Alaska	64.7852433	-141.2567139	1903	41.21	M
USNM	131314	Alaska	64.7852433	-141.2567139	1903	43.65	M
USNM	131320	Alaska	64.7852433	-141.2567139	1903	42.87	M
USNM	131322	Alaska	64.7852433	-141.2567139	1903	41.80	M
USNM	131323	Alaska	64.7852433	-141.2567139	1903	43.40	F
USNM	131326	Alaska	64.7852433	-141.2567139	1903	43.06	M
USNM	131327	Alaska	64.7852433	-141.2567139	1903	42.27	F
USNM	131328	Alaska	64.7852433	-141.2567139	1903	41.75	M
USNM	131329	Alaska	64.7852433	-141.2567139	1903	44.13	M
USNM	131331	Alaska	64.7852433	-141.2567139	1903	41.74	M
USNM	131334	Alaska	64.7852433	-141.2567139	1903	40.55	M
USNM	131335	Alaska	64.7852433	-141.2567139	1903	40.69	F
USNM	131336	Alaska	64.7852433	-141.2567139	1903	41.87	M
USNM	131339	Alaska	64.7852433	-141.2567139	1903	43.48	M
USNM	131340	Alaska	64.7852433	-141.2567139	1903	43.79	M
USNM	131341	Alaska	64.7852433	-141.2567139	1903	42.36	F
USNM	131343	Alaska	64.7852433	-141.2567139	1903	41.31	F
USNM	131890	Alaska	64.7852433	-141.2567139	1903	42.28	F
USNM	134936	Yukon Territory	64.0499992	-139.0833282	1904	43.57	M
USNM	134938	Yukon Territory	64.0499992	-139.0833282	1904	38.68	M
USNM	134939	Yukon Territory	64.0499992	-139.0833282	1904	41.85	M
USNM	148589	Alaska	63.06	-151	1906	44.12	M
USNM	148590	Alaska	63.06	-151	1906	41.43	F
USNM	148591	Alaska	63.06	-151	1906	41.48	F
USNM	148592	Alaska	63.2163051	-150.7351684	1906	43.63	M
USNM	148593	Alaska	63.2163051	-150.7351684	1906	42.99	M
USNM	157225	Alaska	63.4799575	-149.916687	1908	43.79	M
USNM	157226	Alaska	63.4799575	-149.916687	1908	42.61	F
USNM	157227	Alaska	63.4799575	-149.916687	1908	42.73	F
USNM	157228	Alaska	63.4799575	-149.916687	1908	41.09	F
USNM	157229	Alaska	63.4799575	-149.916687	1908	42.77	M
USNM	157230	Alaska	63.4799575	-149.916687	1908	43.44	M
USNM	175128	Alaska	61.3796204	-148.425293	NA	42.63	M
USNM	241741	Alaska	63.4290174	-144.1818237	1921	44.71	M

USNM	241742	Alaska	64.2038918	-142.9988937	1921	42.54	M
USNM	241743	Alaska	64.2886639	-143.1013794	1921	42.99	M
USNM	244057	Alaska	63.7498741	-149.2915344	1923	41.19	F
USNM	271691	Alaska	63.8769714	-147.3355865	1941	42.57	F
USNM	271692	Alaska	64.0449982	-147.6049957	1941	45.37	M
USNM	271693	Alaska	64.0449982	-147.6049957	1941	44.21	F
USNM	271694	Alaska	64.0449982	-147.6049957	1941	43.80	F
USNM	271695	Alaska	64.0449982	-147.6049957	1941	43.77	F
USNM	271697	Alaska	64.0449982	-147.6049957	1941	40.98	M
USNM	512800	Alaska	65.0986137	-143.4677811	NA	44.40	NA
USNM	512802	Alaska	65.1668859	-143.5913085	1975	42.99	F

Appendix B – Resampled site specimens

Measurements taken from specimens collected in 1962 by Robert Spooner near Paxson, AK (63.083, -145.617) and by the author during summer 2007. TL = total body length (mm); a) toothrow length at alveolar margin (mm), b) rostral breadth at infraorbital foramen (mm), c) minimum orbital breadth (mm), d) greatest skull length (mm), e) maximum skull breadth at zygomatic arch (mm), f) breadth at palatal constriction (mm), g) palate width measured at second premolar (mm), h) palate width measured at fourth premolar (mm), i) palate width measured at second molar (mm). Mass and TL measured by respective collector, all other measurements taken with digital calipers.

Catalog No.	Year	Sex	Mass (g)	TL	a	b	c	d	e	f	g	h	i
UAM 7083	1962	F	126.6	164	8.26	5.85	NA	NA	NA	NA	NA	NA	NA
UAM 7107	1962	M	119.3	171	8.02	6	5.23	41.25	21.63	5.38	9.06	11.99	12.55
UAM 7102	1962	F	126.8	176	8.02	6.04	5.65	NA	21.41	5.47	9.21	11.91	12.63
UAM 7089	1962	M	NA	176	8.51	6.06	5.3	43.01	NA	NA	NA	NA	NA
UAM 7101	1962	F	153	181	8.33	6.16	5.84	NA	NA	5.63	9.44	12.36	13.13
UAM 7090	1962	F	NA	162	8.13	6.28	5.36	41.03	21.58	5.01	9.31	11.73	12.35
UAM 7095	1962	M	135	178	8.65	6.35	5.57	42.97	NA	5.25	9.29	12.03	12.6
UAM 102496	2007	F	133.09	161	8.41	5.93	5.32	44.2	21.68	5.63	9.52	12.12	12.55
UAM 102487	2007	NA	133.76	139	NA	6.43	5.61	NA	NA	NA	NA	NA	NA
UAM 102506	2007	F	137.95	153	8.48	6.44	5.44	43.74	22.38	5.57	9.85	12.17	12.62
UAM 102507	2007	M	142.73	182	8.75	6.47	5.65	43.39	22.31	5.42	9.86	12.32	12.85
UAM 102497	2007	F	119.6	153	8.55	6.5	5.5	42.43	22.04	5.33	9.64	12.53	12.62
UAM 102495	2007	M	158.22	152	8.77	6.67	6.1	45.44	NA	5.36	9.86	12.5	12.57

Appendix C – Does *Ochotona princeps* conform to Bergmann's rule?

Smith (1978) included a figure in his manuscript that Ashton et al. (2000) used to infer conformance to Bergmann's rule in the American pika (*O. princeps*). A simple linear regression on the data from Smith (1978) indicated no significant relationship between body size and either latitude or temperature.

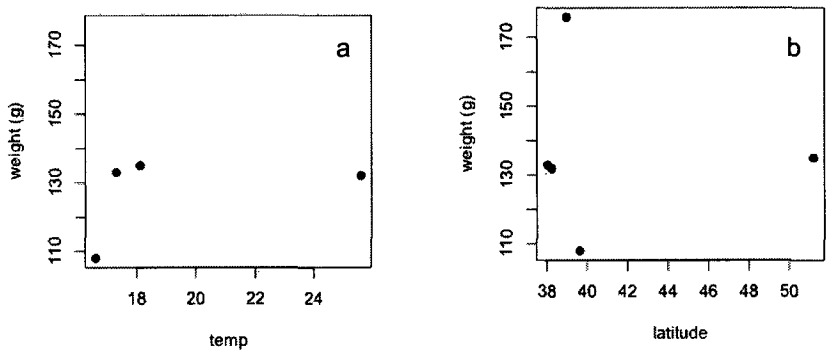

Fig. C-1 – Relationship between mass (g) and temperature (a) or latitude (b) for *O. princeps* in the data presented in Smith (1978).

Chapter 3:

Phylogeography of an arctic-alpine mammal, the collared pika (*Ochotona collaris*)[1]

3.1 Abstract

Pikas (*Ochotona* spp.) are alpine specialists that show considerable geographic variation structured along latitudinal and altitudinal gradients. Recent phylogeographic studies of the mid-latitude American pika (*O. princeps*) in North America and the black-lipped pika (*O. curzoniae*) in east Asia indicate phylogeographic structuring among separate mountain ranges and drainages. Theory predicts that lower genetic diversity and less phylogeographic structure will exist at higher latitudes due to lower long-term habitat stability. We studied the phylogeographic structure of collared pikas (*O. collaris*) throughout their known distribution in Alaska and northwestern Canada using the mitochondrial cytochrome *b* gene. Our analyses revealed two geographically separate and genetically divergent haplogroups: one in the Wrangell-St. Elias and Coast mountain ranges, and the other throughout the rest of their range (from Lake Clark to the Northwest Territories). The phylogeographic subdivision may be the result of localized differentiation in cryptic refugia during the Pleistocene. Alternatively, they may have been separated in sub-Laurentide refugia prior to subsequently dispersing north. Population genetic analyses revealed statistically significant differentiation between most populations of *O. collaris*, with only four haplotypes shared between populations. Variation was mostly structured within populations

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[1] Lanier, H.C. and L. E. Olson. 2010. Phylogeography of an arctic-alpine mammal, the collared pika (*Ochotona collaris*). Prepared for submission to the *Journal of Mammalogy*.

(as opposed to between populations or mountain ranges). When within-species nucleotide diversity in *O. collaris*, *O. princeps*, and *O. curzoniae* populations are compared, diversity declined linearly with increasing latitude. Populations of *O. collaris* are less geographically structured and more closely related to one another than populations of either *O. princeps* or *O. curzoniae.* Collared pikas are currently considered to be a common species at low risk of extinction, but their precarious thermal tolerance, narrow ecological niche, high degree of population isolation, and low genetic variation may have consequences for their continued viability in a warming world.

3.2 Introduction

The latitudinal gradient in diversity is one of the oldest recognized patterns in biogeography (Turner and Hawkins 2004), with more species, subspecies, and genetic diversity found at lower latitudes (Wiens and Donoghue 2004). On a phylogeographic level, much of this pattern has been attributed to niche conservatism, the length of regional habitat occupancy, and the extent of inhabitable area over time (Carnaval et al. 2009; Martínez-Meyer et al. 2004; Wiens and Donoghue 2004). Large-scale Quaternary phenomena in northern regions, such as ice sheets covering much of northern North America during the Pleistocene, have also played a formative role (Hewitt 2004; Lessa et al. 2003). Areas that were glaciated during the Pleistocene contain lower genetic diversity than unglaciated areas, especially those with a long history of occupation (Hewitt 2000). While broad-scale latitudinal patterns of genetic diversity have been widely studied for temperate species, many cold- and alpine-adapted species show inverse trends, with relatively greater genetic and species diversity at higher latitudes (Arnason et al. 2006; Dalén et al. 2004; Fedorov and Stenseth 2002; Melo-Ferreira et al. 2007).

For montane species, elevation also plays an important role in structuring genetic diversity. Elevational gradients in temperature and area (both decrease at higher altitudes; Körner 2007) can isolate alpine populations and species into separate mountain regions (Brown 1971), and the degree of topographic isolation can vary latitudinally. Additionally, abiotic barriers to dispersal between lower latitude mountains are thought to be greater than those between higher latitude mountains (Ghalambor et al. 2006; Janzen 1967). Evolutionary processes affected by these barriers have the potential to highly structure montane biodiversity at lower latitudes, whereas high latitude montane populations may be less structured. Biotic connections between mountain ranges have been further impacted by Quaternary climate change. Montane glaciations shifted alpine habitat and climate

to lower elevations during glacial periods, allowing contact among previously disjunct interglacial populations (Galbreath et al. 2009; Knowles 2000). Connections between disjunct mountain ranges may be stronger at higher latitudes, where Quaternary glaciations were more extensive and elevational zonation is less extreme (Körner 2007; Pielou 1991).

Pikas (*Ochotona*) are one group of alpine specialists in which geographic variation is structured along latitudinal and altitudinal gradients (e.g., Galbreath et al. 2009). These small-bodied members of the mammalian order Lagomorpha (rabbits, hares, and pikas) are territorial, non-hibernating herbivores notable for their high intraspecific and low interspecific variation (Corbet 1978; Hoffmann and Smith 2005; Smith 2008). *Ochotona* is thought to have originated and diversified in Asia (Erbajeva 1994) and is represented in North America by two species, the American pika (*O. princeps*) and the collared pika (*O. collaris*; Figure 1) Sensitivity to high temperatures is currently thought to restrict *O. princeps* to "sky islands" in the Intermountain West (Beever et al. 2003; MacArthur and Wang 1973). However, during the Pleistocene, *O. princeps* was more widespread in North America (Grayson 2005; Kurtén and Anderson 1980; Mead 1987). Phylogeographic and ecological studies have revealed recent distributional shifts toward higher elevations and latitudes (Galbreath et al. 2009). The five main phylogeographic groups occurring in *O. princeps* are broadly structured into separate mountain regions that exhibit population differentiation influenced by Pleistocene landscape features (Galbreath et al. 2009; Galbreath et al. 2010; Hafner and Sullivan 1995).

Much less is known about the northernmost of the two Nearctic species, the collared pika (*O. collaris*). In E. R. Hall's (1981) classic work, *The Mammals of North America*, 36 distinct subspecies and a range map showing a number of major discontinuities are provided for *O. princeps* (Figure 1). The treatment of *Ochotona collaris*, then considered a subspecies of *O. princeps*, was based on 14 museum specimens, much less detailed, and implied a geographically continuous

distribution. In 1981, *O. princeps* was represented by nearly six times as many specimens as *O. collaris* (2408 to 403, respectively, Figure 1). That gap has only widened in recent years. In terms of published literature, Web of Science searches for *O. collaris* publications turned up approximately a quarter of the papers recovered for *O. princeps* (20 and 88, respectively; ISI Web of Science keywords "*Ochotona*" AND "*princeps*" or "*Ochotona*" AND "*collaris*" on 2 Feb 2010). This lack of knowledge is not a trifling matter. Poor knowledge of occurrence and lack of clarity in range maps can lead to errors of commission (false occurrences) in determining a species' occurrence (Gaston and Fuller 2009). For example, several publications list possible occurrences of *O. collaris* in the Brooks Range of northern Alaska (reviewed in MacDonald and Cook 2009), but these accounts have never been verified with a specimen, scat, or even a photograph. Historical data for *O. princeps* populations have been used to track and model climate-mediated extirpation (Beever et al. 2003; Beever et al. 2010), whereas such precise locality information is unavailable for nearly all *O. collaris* specimens collected during the last millennium. However, the disappearance of historically known populations at the southern range margins has long been noted (e.g., Swarth 1936). Distinguishing climate-mediated decline from metapopulation dynamics (Morrison and Hik 2008) may be important for understanding the effects of climate change on *O. collaris.*

More recently, *O. collaris* has been the subject of several ecological and behavioral studies in a limited portion of their range (Franken and Hik 2004b; MacDonald and Jones 1987; Morrison and Hik 2007, 2008; Smith et al. 2004; Trefry and Hik 2009a). These studies suggest that *O. collaris* is highly philopatric but is subject to repeated extinction and recolonization events between habitat patches within a site (Franken and Hik 2004a; Franken 2002). There has been little geographic differentiation characterized throughout their range, although Baker (1951) suggested the possibility of morphological differentiation between Canadian and Alaskan collared pikas. Population variability in call structure has been

described, with 94% of individuals being correctly classified by population on the basis of call (Trefry and Hik 2009b). Fossil data indicate that *O. collaris* was present in Pleistocene Beringia, the ice-free corridor connecting North America and Asia (Guthrie 1973; Weston 1979). *Ochotona collaris* is thought to have been geographically, although perhaps not temporally, sympatric with the larger †*O. whartoni*, which appears to have independently crossed the Bering Land Bridge into eastern Beringia (Weston 1979). Guthrie (1973) hypothesized the allopatric divergence of *O. collaris* from a common ancestor shared by the other North American species, *O. princeps*, in separate Pleistocene refugia. While the Nearctic species may have diverged in allopatry, recent studies suggest that their divergence predated the Pleistocene (Galbreath et al. 2010; Lanier and Olson 2009).

We have three objectives for this study: first, to characterize the phylogeographic structure and history of *O. collaris*; second, to determine recent local and range-wide demographic trends within *O. collaris*; and third, to compare the observed diversity and differentiation within *O. collaris* to phylogeographic patterns that have been recently inferred in other species of pika (Ci et al. 2009; Galbreath et al. 2009).

3.3 Methods

3.3.1 Sampling strategy and study area

The interrelated challenges of accessibility and affordability are partly responsible for the relative lack of range-wide research on *O. collaris*. Field work on pikas is challenging, as many of the regions they inhabit are not road-accessible. This problem is exacerbated in northwestern Canada, where few museum specimens of mammals have been collected since the advent of modern molecular techniques. In order to increase the geographic range of our sampling, particularly into Canada, we included samples taken from historically collected museum specimens. The

incorporation of historical specimen material into phylogeographic and phylogenetic studies is becoming more common (Debruyne 2005; Olson et al. 2005; Ramey et al. 2005). While not strictly ancient, these sources of degraded or "antique" DNA are subject to many of the same risks as far as contamination and chimerism (Olson and Hassanin 2003). "Antique" samples from 27 museum specimens included in this study ranged from 30 to 120 years old (Figure 1). Locality information was recorded from specimen tags. When information on latitude and longitude was not recorded by the collector, it was determined using the Berkeley Georeferencing Guidelines BioGeomancer (http://bg.berkeley.edu/latest/) and the Natural Resources Canada gazetteer (http://atlas.nrcan.gc.ca/). Museum catalog numbers, population designation, and locality information are given in the appendix.

Field collecting was conducted on Alaskan populations of *O. collaris* during the summers of 2005-2008. Samples of heart, kidney, or muscle were retained for analysis in buffer or sampled directly into Cell-Lysis Solution (PureGene, Gentra Systems Inc.) in the field. Additional tissues from previously collected specimens were subsampled from specimens housed in the University of Alaska Museum Mammal Collection (http://arctos.database.museum/). A map showing the collection localities of all specimens included in this study is provided in Fig. 2, with additional information in Table 1.

3.3.2 Laboratory methods

We amplified 1140 bp of the mitochondrial cytochrome *b* gene from 148 modern samples using the primers CB-HLF1 and CB-HLR1 (Table 2) and sequenced the resulting product using a combination of those primers and CB-HLiF2 and CB-HLiR2 (Lanier and Olson 2009). Procedures for extraction and amplification are detailed in Lanier and Olson (2009).

For the historical specimens, fragments of dried tissue (ca. 0.5-5 mm^3) were

removed from skulls or skeletons. Prior to extraction, samples were washed in 100% ethanol overnight, 70% ethanol for three hours at 55°C, sdH2O at room temperature for three hours, and sdH2O overnight at room temperature (protocol modified from Olson et al. 2005). Larger samples were extracted using the PureGene Kit (Gentra Systems, Inc) following the Animal Tissue Protocol (omitting the RNAse). Additional Proteinase-K (PureGene kit, Gentra Systems, Inc) was added every 24 hours until tissues were fully digested. Extracted DNA was resuspended in 50 μL DNA Hydration Solution (Gentra Systems, Inc) and diluted 1:10 for PCR. For smaller samples (< 1 mm^3), the QIAamp DNA Micro Kit (Qiagen) was used following the tissue sample protocol. Extracted DNA was resuspended in buffer and diluted 1:10 for PCR.

Specialized protocols were followed to minimize the possibility of contamination of DNA extracted from degraded museum samples. Concatenation of multiple short fragments to reconstruct longer sequences is fraught with risk, especially in the case of degraded DNA (Olson and Hassanin 2003). All extractions and PCR set-up were conducted in the University of Alaska Museum's Ancient DNA Facility, which resides in a PCR-free building. Each sample was amplified in nine different PCR reactions to yield fragments of 215-272 bp with 2-3x coverage over most of the gene (Figure 3). Samples were amplified in 15 μL volumes using regular 7.5 μL GoTaq Green Mastermix (Promega), 1.2 μL diluted template, 3.9 μL ultrapure water, and 1.2 μL each of the primer pairs listed in Table 2. Amplification followed standard PCR procedures (Lanier and Olson 2009), with 44 cycles of denaturating, annealing, and extension with annealing temperatures ranging from 48-55°C. Each PCR was run with extraction negatives, PCR negatives, and a positive antique DNA control. This approach was successful for historically collected samples regardless of their age; a linear regression indicated no relationship between year of collection and success of PCR for the antique material ($p = 0.1711$).

All PCR products were cleaned using 1.38 μL ExoSap (USB Corp) with a 1:2:4 ratio of Exo:Sap:10X dilution buffer. Samples were cycle sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) in 10 μL reaction volumes (1 μL template, 0.6 μL primer, 0.5 μL big dye, 1.5 μL 5X buffer, and 6.4 μL water) following the manufacturer's instructions. Sequencing products were purified with Sephadex G-50 fine clean up. Sequencing was conducted on an ABI 3100 Genetic Analyzer (Applied Biosystems) in the Institute of Arctic Biology Core Facility for Nucleic Acid Analysis of the University of Alaska Fairbanks. Sequences were inspected, edited and aligned in Sequencher 4.6 (Gene Codes Corp.) and verified by eye.

3.3.3 Sequence verification

We used resequencing and analytical methods to rule out the possibility of investigator error and inadvertent chimerism. Approximately one-third of the fresh samples were amplified and sequenced twice for verification. All antique samples were amplified and sequenced in separate reactions, with overlapping polymorphic sites wherever possible. In addition, a 250 bp sliding window approach indicated no relationship between gene region and reticulation (as would be expected if the reticulation was the result of chimerism). The reticulation appears to be the result of true homoplasy in our dataset, although it is not clear whether it results from parallel mutations or reversals.

3.3.4 Phylogenetic analyses

To examine the ancestral relationships between individuals within *O. collaris*, we first used a traditional phylogenetic framework. Tree-building methodologies have a long history in phylogeographic studies and can reveal information about the genetic distance between haplotypes and the statistical support for those relationships (Avise 2009). The lack of resolution in phylogenetic trees can also be informative regarding lineage sorting and for historical demographic inference. The

Akaike Information Criterion (AIC) was used in jModelTest (Guindon and Gascuel 2003; Posada 2008) to select the most appropriate model of nucleotide substitution. We then implemented this model in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Tree searches were conducted with four chains (three of which were heated). Analyses were allowed to proceed for 10 million generations with sampling every 1000 generations. The first 200 trees were discarded as burn-in using a plot of -ln L scores against generation as a guide.

We also inferred intraspecific relationships in our dataset by constructing statistical parsimony haplotype networks in TCS (Clement et al. 2000) and split decomposition networks in SplitsTree (Huson and Bryant 2006). These methods can be particularly useful for revealing geographic structure when phylogenetic trees are poorly resolved and when ancestral haplotypes (sequences identical to those that gave rise to daughter sequences, both in a contemporaneous sample) are present in the dataset (Posada and Crandall 2001).

3.3.5 Population assignment

We assigned populations based on minimum geographic proximity between sampling localities and maximum distance between clusters of samples (Figure 1). Higher geography was assigned by mountain range (Table 1; Figure 2). Seven isolated localities were represented by fewer than four individuals; these localities were only used in the regional comparisons. Based on levels of population differentiation from other phylogeographic studies of Alaskan mammals using mtDNA (e.g., Eddingsaas et al. 2004; Fedorov et al. 2003; Weksler et al. accepted), further population subdivision is unlikely to present an unrecognized bias in our analyses.

3.3.6 Population genetic analyses

To examine the geographic subdivision of genetic diversity and compare diversity levels between populations we used a series of standard population

genetic summary statistics computed in DnaSP v.5 (Librado and Rozas 2009). These statistics allow a comparison of genetic diversity within *O. collaris*, and with other studies, although they distill variation down to several metrics disregarding information on the genealogy of alleles. We tested for population demographic fluctuations to look for evidence of post-Pleistocene expansion (Hewitt 2004) and/or recent population decline (similar to those that have been described for *O. princeps*; Beever et al. 2010). We used R_2 (Ramos-Onsins and Rozas 2002), Fu's *Fs* (Fu 1997), and Fay and Wu's *H* (Fay and Wu 2000) to assess historical demographic change. The R_2 of Ramos-Onsins and Rozas (2002) is based on the difference between the number of singleton mutations and the average number of nucleotide differences. Fu's *Fs* (Fu 1997), a metric that compares a coalescent estimator of the haplotype frequency distribution based on a nucleotide diversity estimator of theta (θ_π, the effective population size scaled by the mutation rate), and R_2 have the most power to detect population growth (Ramos-Onsins and Rozas 2002). Both *Fs* and R_2 values in the American pika have been previously published (*O. princeps*; Galbreath et al. 2009), making them useful comparative statistics. Fay and Wu's *H* (2000) contrasts low and intermediate frequency alleles, making it sensitive to population decline and population subdivision (Zeng et al. 2006). Negative values of Fay and Wu's *H* and Fu's *Fs* and small positive values of R_2 can be indicative of sudden population expansion. Significantly positive values of *Fs* and *H* can indicate population contraction (Zeng et al. 2006). Significance for each of these tests was calculated using 10,000 coalescent simulations in DnaSP. We also calculated mismatch distributions in DnaSP for each mountain region and for the population as a whole to contrast pairwise genetic differences with those expected under a model of sudden demographic expansion (Harpending et al. 1998). To look at geographic subdivision among populations and mountain ranges we used an Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) and calculated population

pairwise Φst statistics in Arlequin v.3.1 (Excoffier et al. 2005). Significance was tested with 1,000 permutations in Arlequin.

3.4 Results

We recovered moderate haplotype diversity in *O. collaris,* with 79 unique haplotypes for 173 individuals and an *Hd* of 0.983 (Table 3). Of those samples, 27 were from historic specimens. For the whole dataset, there were 115 polymorphic sites, of which 85 were synonymous and 47 were singleton mutations. The historic dataset matched the overall profile for the rest of the dataset, with 21 haplotypes and 48 variable sites. The per-site nucleotide diversity (π) was 0.007 for the entire dataset and 0.0067 for the antique samples. The general time-reversible model (GTR) with a proportion of invariant sites (I) and a gamma rate parameter (G) was selected as the most appropriate model of evolution for the entire dataset.

One major phylogeographic discontinuity was present: a strongly supported clade was recovered in the southern regions of the range in the Wrangell-St. Elias and Coast mountain ranges (populations k, l, m, and n as well as individuals from j and f). The rest of the genealogical relationships among samples were unresolved (hereafter referred to as the Beringian lineage to distinguish it from the Southern Canadian lineage). Phylogenetic analyses lacked broad-scale phylogeographic resolution for much of the range of collared pikas, with most samples forming a basal polytomy (Figure 4). This is not unexpected for comparisons at this scale of divergence (e.g., Weksler et al. accepted) but can be important when compared with other codistributed or closely related species.

Most populations in our study were significantly differentiated from one another based on Φst (Table 4). Populations from the Northwest Territory (population o) were not significantly different from those in the Ogilvie Mountains (population i). Differentiation was not statistically significant between populations

with low sample sizes (e.g., Richardson Mountains and Denali), although this may be an artifact of reduced statistical power due to low sample size. This lack of statistical significance may have little biological meaning. Based on pika dispersal capability (Peacock and Ray 2001) and the other Φst results, it is highly unlikely that these populations are exchanging migrants. Haplotype sharing between populations was also low, providing additional evidence for low migration between populations. We recovered four haplotypes shared between populations, two of which were also shared between mountain regions. Results from the AMOVA (Table 5) further support this pattern, as the majority of the variation is partitioned within populations (48.78%) or among populations within a range (32.69%). Relatively little genetic variation is partitioned among mountain ranges (18.53%).

The mismatch distribution, R_2, and Fu's *Fs* all indicate past population expansion in *O. collaris* (Table 3). This finding was in contrast to our findings of demographic stability within most groups at the mountain range and population level. Only the Mackenzie Mountains (and within them the Northwest Territories; population o) exhibited a statistically significant R_2. Values of Fu's *Fs* indicated demographic decline in the eastern Chugach (population f), but neither summary statistic indicated population decline in this region. Fu's *Fs* contrasts a neutral coalescent estimate of the expected number of alleles based on θ_π (a nucleotide diversity estimator of the θ) against the actual number of alleles (Fu 1997). Because the Beringian and Southern Canadian haplogroups occur in sympatry in this region (inflating the estimate of θ_π), this result likely reflects population subdivision instead of demographic decline.

3.5 Discussion

3.5.1 Phylogeography of *O. collaris*

There is one major phylogeographic discontinuity evident within *O. collaris*. A well-supported, parapatric subclade (> 0.90 posterior probability) is found in the

Wrangell-St. Elias and Costal mountain ranges. This clade extends from northwest BC into southwest Yukon Territory, and encounters the Beringian lineage in southcentral Alaska (in southern Wrangell-St. Elias National Park and Preserve). Differentiation based on Φst between the two major haplogroups ranged from 0.57 to 0.81, exceeding most of the within-haplogroup Φst values. This subdivision may have resulted from the Pleistocene isolation of pika populations in ice-free cryptic refugia, separate from those in central Beringia (as described in Loehr et al. 2006; Pielou 1991; Stewart et al. 2010). Suggestions of localized cryptic endemism in several species support this hypothesis. Phylogeographic studies of singing voles (*Microtus miurus*; Weksler et al. accepted) have revealed a highly divergent lineage occurring in the Wrangell Mountains. A mitochondrially divergent, putatively distinct species of deer mouse (*Peromyscus* sp.; Lucid and Cook 2007) has been suggested from southwest Yukon Territory. Neither of these studies incorporated dense geographic sampling, so the geographic extent of the lineages is unknown. The percent divergence between the two phylogroups in collared pikas (0.6%) is less than those described for singing voles or deer mice (4.2% and 4.32%, respectively). This might be a reflection of lower effective population size and/or slower mutation rates in collared pikas.

Alternatively, this pattern might result from secondary contact occurring between lineages isolated in the separate Beringian and sub-Laurentide refugia during the Pleistocene. For several species, the Wrangell-St. Elias region represents a contact zone between divergent (but not locally endemic) lineages. Widespread phylogroups in brown lemmings (*Lemmus trimucronatus*; Fedorov et al. 2003) and ermine (*Mustela erminea*; Fleming and Cook 2002) come into contact in Wrangell-St. Elias, although different refugial histories have been proposed for each species. The presence of contact zones for multiple species may be indicative of a shared phylogeographic history of vicariance and isolation.

Strong population differentiation in pikas is expected given their degree of philopatry (Peacock and Ray 2001), and our results indicate a high degree of geographic partitioning at the within-population level. Low haplotype sharing was further indicative of population differentiation. Low nucleotide diversity and high haplotype diversity are considered to be evidence of a strong population bottleneck followed by population growth and the accumulation of mutations (Grant and Bowen 1998). This pattern is also likely to translate to low resolution of phylogenetic relationships; relationships among each phylogroup were poorly resolved on all but local levels (Figures 4 and 5). Strong past population growth followed by population subdivision can result in a pattern of localized differentiation with little broad-scale geographic partitioning (similar to *Lepus timidus*; Melo-Ferreira et al. 2007). Pleistocene population expansion may have been aided by widespread glaciations in southern Alaska and northwestern Canada. Collared pikas are commonly found in talus above treeline (MacDonald and Jones 1987), and early successional stages (enhanced rocky substrate, herbaceous plants, reduced woody vegetation) along glacial margins may have enhanced dispersal. Glacier margins and termini often support populations of *O. collaris* (pers. obs). There are possibilities of dispersal across glaciers as well; however, the success of this venue is unknown. Collared pikas living on nunataks have been observed to venture kilometers onto glaciers (S. Koh, pers. comm.) whereas individuals dwelling on talus slopes will forage only a few meters into the meadow (Morrison et al. 2004). Glacially mediated dispersal of either sort might be reflected in the distribution of lineages in Wrangell-St. Elias NPP. The Southern Canada clade was better represented in the eastern Chugach region (population f) of southern Wrangell-St. Elias NPP than in the northern regions of the park (in the Wrangell Mountains; population j). The Bagley and the St. Elias icefields harbor nunatak and glacial margin populations of pikas (Cook and MacDonald 2003; Murray and Murray 1969) that could contribute to gene flow into this region. Future studies of icefield-

mediated gene flow in pikas and other codistributed alpine mammals may be interesting from both historical biogeography and conservation perspectives.

3.5.2 Comparison to other phylogeographic studies in pikas

Although the number of described subspecies (36; Hall 1981) in American pikas may be upwardly biased by historical research, our results indicate that most populations we sampled are genetically differentiated. Historical factors (glaciations and time since population expansion) have played a strong role in shaping the geographic distribution of diversity (or lack thereof) in collared pikas. *Ochotona princeps* is composed of five well-supported mtDNA phylogroups, with divergences between groups ranging from 1.6-2.7% (Galbreath et al. 2009). Only two geographically distinct haplogroups were evident in *O. collaris*, and the uncorrected sequence divergence (0.6%) between them was less than half that in *O. princeps*. Greater phylogeographic divergences have also been described in the black-lipped pika (*O. curzoniae*; Ci et al. 2009) from the Tibetan Plateau. Whereas the majority of genetic variance was explained by within-population differences in *O. collaris*, among-group structuring explained the majority of the variance in *O. curzoniae* (Ci et al. 2009). Populations of collared pikas are less geographically structured and more closely related to one another than are populations of either American pikas or black-lipped pikas.

Nucleotide diversity in pikas declined at higher latitudes (Figure 6), broadly fitting the southern diversity, northern purity model (described by Hewitt 2004, 1996). However, within any of the three species alone, nucleotide diversity did not significantly decline at higher latitudes. This pattern can result from the increase in genetic drift at distributional limits (Herrera and Bazaga 2007) via low population size, low survivorship, and/or recent colonization (Hampe and Petit 2005). Decline and extirpation documented in lower-latitude populations of *O. princeps* (Beever et al. 2003; Grayson and Livingston 1993) is likely to reduce genetic diversity in those

populations over time (Allendorf and Luikart 2007). Climate-mediated latitudinal shifts in diversity could lead to a parabolic distribution of within-species diversity, as diversity will be reduced along the leading edge by colonization dynamics and along the lagging edge by localized extinctions (Hampe and Petit 2005; Hewitt 2004). From a demographic perspective, dynamics in *O. collaris* and *O. princeps* bear the most similarity to one another. Summary statistic approaches don't indicate significant population fluctuations in populations or lineages of either Nearctic species (Galbreath et al. 2009; this study). Multiple populations of *O. curzoniae* showed evidence of recent rapid expansion (Ci et al. 2009).

3.5.3 Implications and future directions

"Rear edge" populations, populations occurring at lower-latitudinal or lower-elevational limits of a species range (sensu Hampe and Petit 2005), may be disproportionally important for conservation and evolution because they are anticipated to contain the oldest lineages and be the best adapted to warmer climates. There was reduced genetic diversity but no statistically significant signal of population decline in populations of collared pikas from the southernmost range margin. This might reflect a lag between census and effective population sizes, as older alleles may not have had time to be purged by genetic drift. Despite documented declines along the southern margin of *O. princeps*, mtDNA summary statistics failed to reveal a signal of population decline (Galbreath et al. 2009). The only long-term study of any collared pika population has documented climate-mediated decline in a southwest Yukon Territory site (Morrison and Hik 2007). Resurveys of sites in the Coast Mountains at Atlin Lake (Swarth 1936) and White Pass (Lanier, unpublished data) failed to recover historically documented populations. Distinguishing whether these patterns correspond to climate-mediated declines or metapopulation dynamics (Morrison and Hik 2008) will be critical for predicting the effects of global warming on *O. collaris*.

3.5.4 Conclusions

Based upon the amount of genetic differentiation between regions, we conclude that greater historical connectivity between regions have resulted in less genetic structure within *O. collaris* than found elsewhere. The amount and degree of phylogeographic structure is thus greater for pika species that occur in temperate mountain regions. Current and historical climatic factors and habitat gradients likely have combined to decrease differentiation between *O. collaris* populations. The relative paucity of described subspecies in *O. collaris* is not just a difference in the amount of historical research; it is also the result of historical factors that have acted to diminish genetic diversity throughout the species' range. Compared to other codistributed mammal fauna, *O. collaris* exhibits a similar phylogeographic discontinuity but a lower genetic distance between phylogroups. The IUCN currently considers *O. collaris* to be a common species at low risk of extinction, but their high degree of population isolation and low observed genetic variation may present challenges to their ability to adapt (Reed 2004; Reed and Frankham 2003; Willi et al. 2006). It remains to be seen whether this low diversity will inhibit the ability of *O. collaris* to adapt to habitat and climate change.

3.6 Acknowledgements

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3.7 Figures

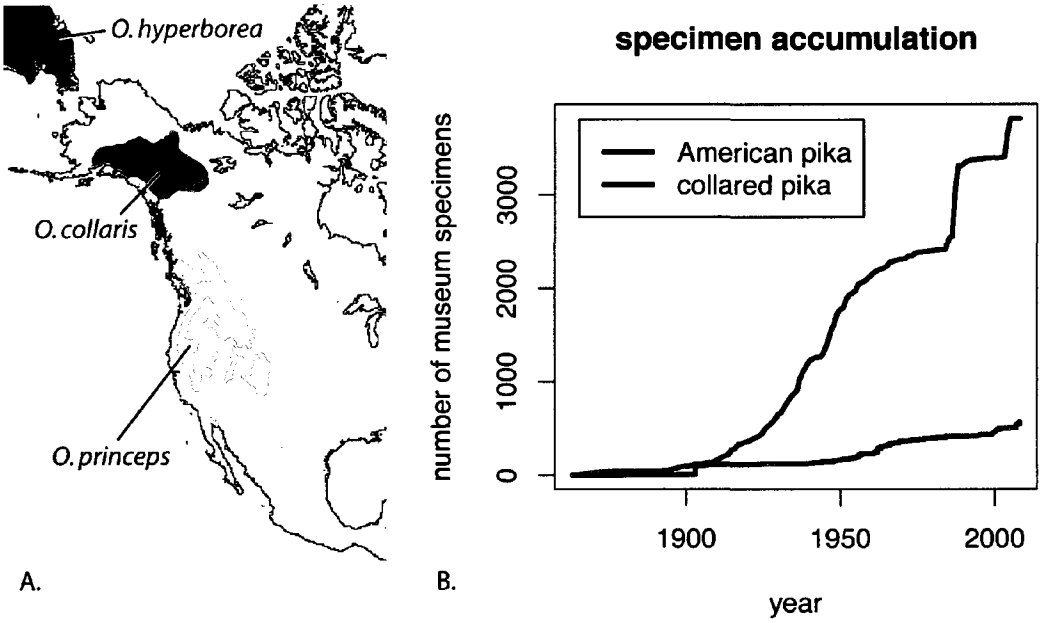


Figure 1 – Range maps and collected specimens of North American pikas

A. Range maps (modified from Hall 1981, MacDonald and Jones 1987, Smith et al. 1990) showing the differences in locality and resolution in the ranges of American pikas and collared pikas. The eastern edge of the range of the northern pika (*O. hyperborea*) is also shown. B.) Accumulation curves showing the disparity between the number American and collared pika specimens in North American museum collections (data obtained from MaNIS; http://manisnet.org/)

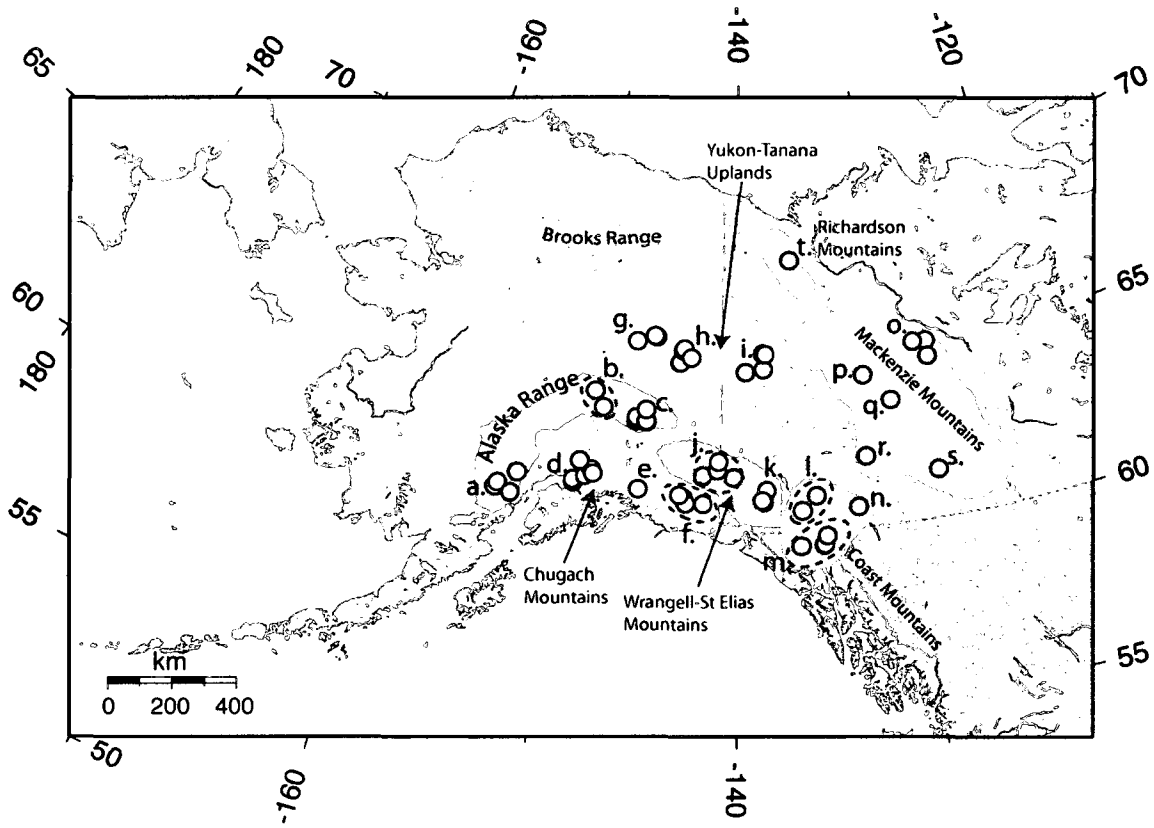


Figure 2 – Sampling map

Localities included in this analysis (localities *a-t* are detailed in Table 1). The major mountain ranges and overall topography of the region is shown behind samples. The majority of the Canadian localities are represented by antique DNA samples from museum specimens (grey circles) whereas fresh material was available for most Alaskan localities (white circles).

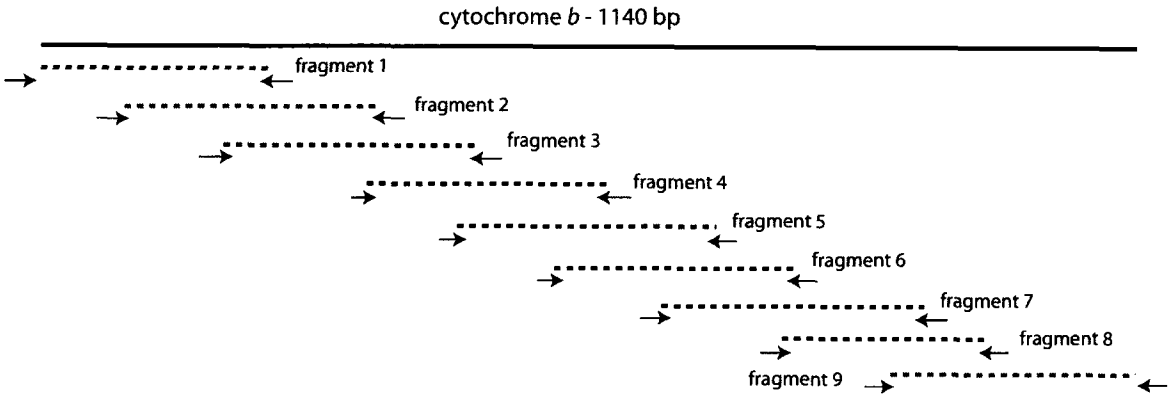
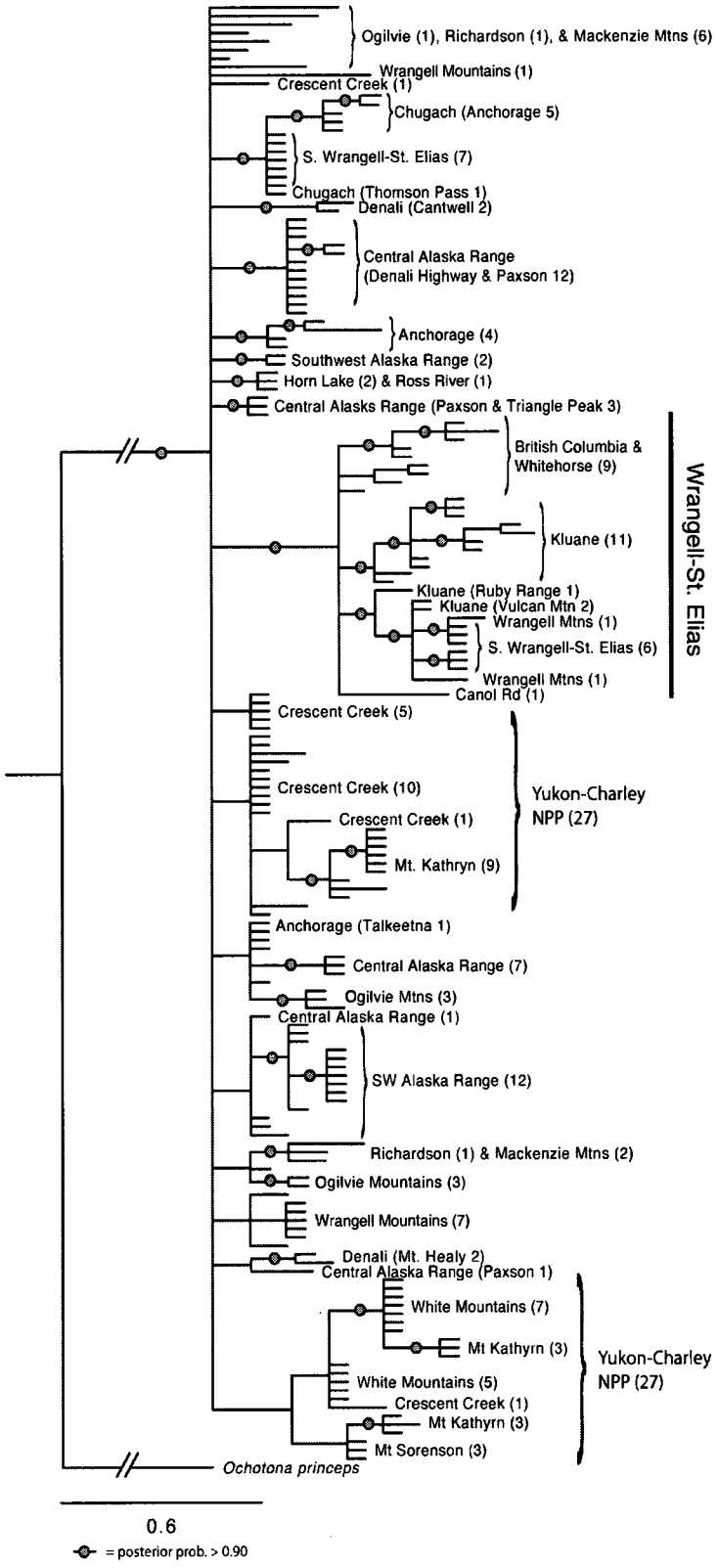


Figure 3 – Antique DNA amplification strategy

Samples were amplified in nine separate PCR reactions and unidirectionally sequenced using the primers in Table 2 to yield a high degree of overlap between all fragments (dotted line) for complete coverage of the cytochrome *b* gene.

Figure 4 – Phylogenetic tree

Majority rule consensus phylogenetic tree for all *O. collaris* samples, with branch leading to *O. princeps* outgroup shortened. Nodes with posterior probability greater than 0.90 are indicated with a grey circle; numbers in parentheses refer to number of samples.


Ogilvie (1), Richardson (1), & Mackenzie Mtns (6)
Wrangell Mountains (1)
Crescent Creek (1)
Chugach (Anchorage 5)
S. Wrangell-St. Elias (7)
Chugach (Thomson Pass 1)
Denali (Cantwell 2)
Central Alaska Range
(Denali Highway & Paxson 12)
Anchorage (4)
Southwest Alaska Range (2)
Horn Lake (2) & Ross River (1)
Central Alasks Range (Paxson & Triangle Peak 3)
British Columbia &
Whitehorse (9)
Kluane (11)
Kluane (Ruby Range 1)
Kluane (Vulcan Mtn 2)
Wrangell Mtns (1)
S. Wrangell-St. Elias (6)
Wrangell Mtns (1)
Canol Rd (1)
Wrangell-St. Elias
Crescent Creek (5)
Crescent Creek (10)
Crescent Creek (1)
Mt. Kathryn (9)
Yukon-Charley
NPP (27)
Anchorage (Talkeetna 1)
Central Alaska Range (7)
Ogilvie Mtns (3)
Central Alaska Range (1)
SW Alaska Range (12)
Richardson (1) & Mackenzie Mtns (2)
Ogilvie Mountains (3)
Wrangell Mountains (7)
Denali (Mt. Healy 2)
Central Alaska Range (Paxson 1)
White Mountains (7)
Mt Kathyrn (3)
White Mountains (5)
Crescent Creek (1)
Mt Kathyrn (3)
Mt Sorenson (3)
Yukon-Charley
NPP (27)
Ochotona princeps
0.6
= posterior prob. > 0.90

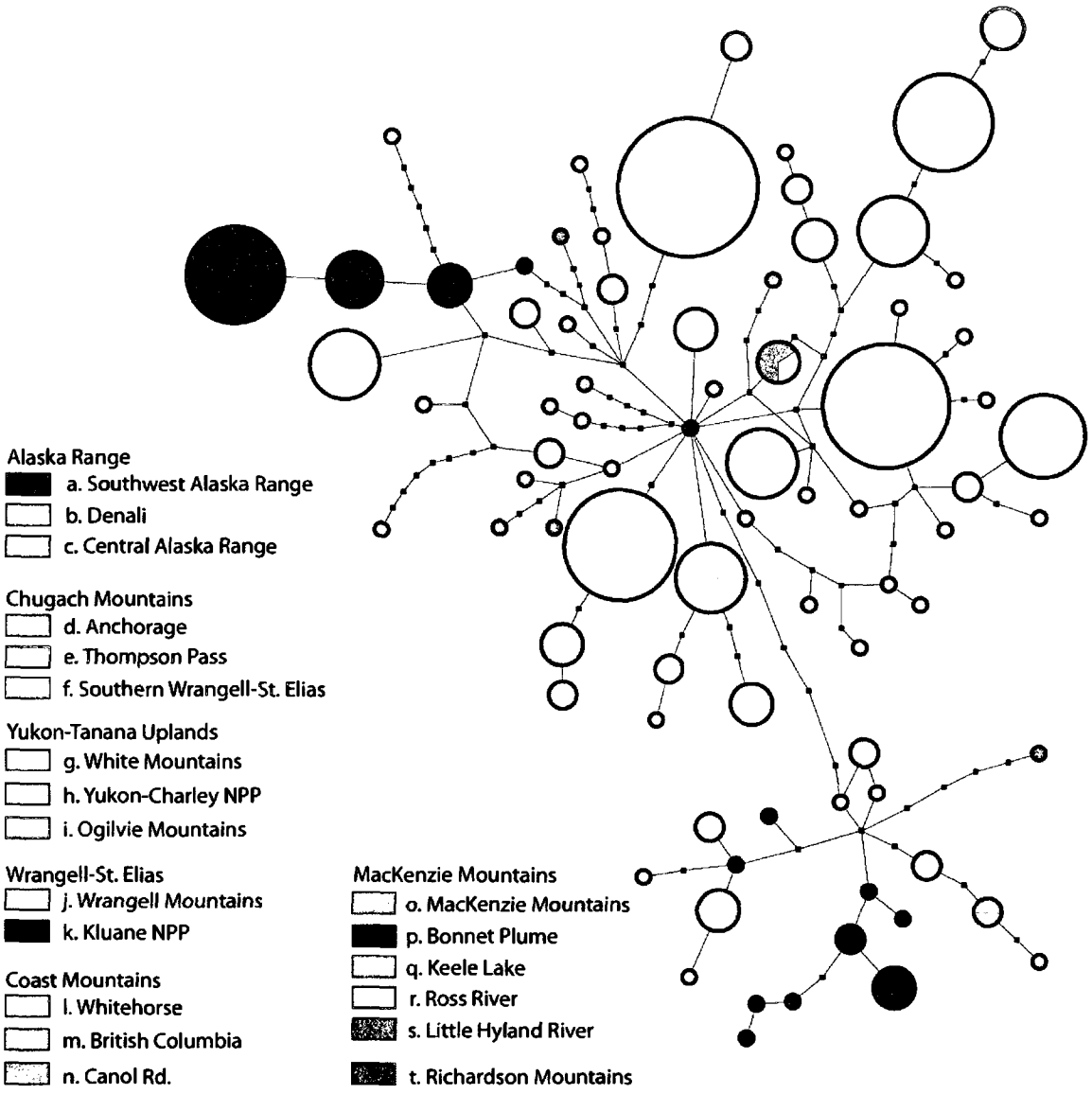


Figure 5 – Haplotype network

Statistical parsimony network of the haplotypes found in *O. collaris*. The Beringian haplogroup is separated by 5 mutations from the Wrangell-St. Elias clade (below). Nodes are proportional to the number of individuals sharing a haplotype (smallest circles correspond to one individual). Square nodes represent unsampled (inferred) haplotypes. Each branch is equal to one mutation. The splits-tree network (not shown) recovered similar structure.

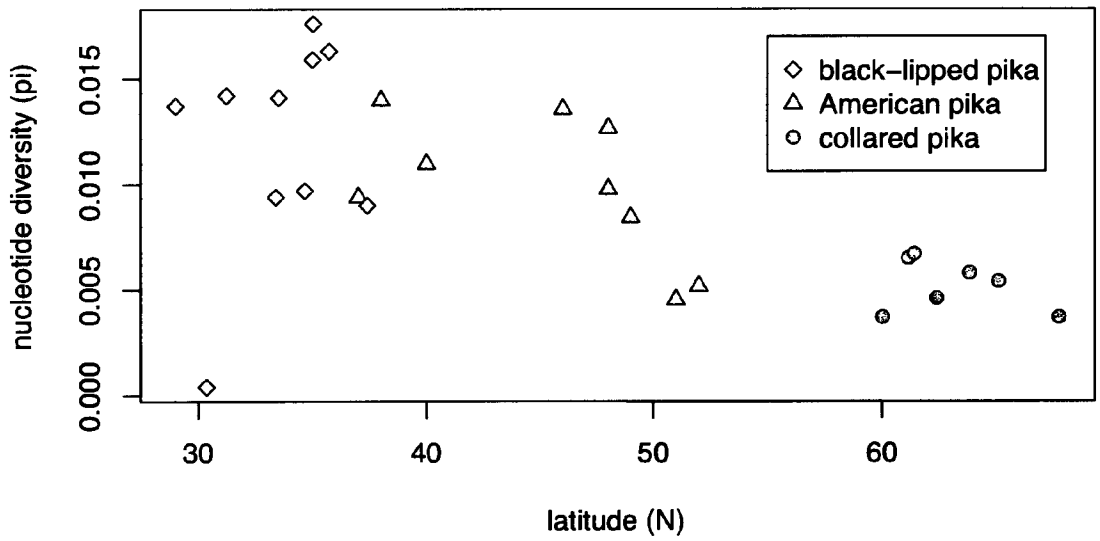


Figure 6 – Latitudinal gradient in nucleotide diversity (π)

Nucleotide diversity for the major regional groups in the blacked lipped pika (*O. curzoniae*; Ci et al. 2009), the American pika (*O. princeps*; Galbreath et al. 2009), and the collared pika (*O. collaris*; this study) are plotted against the average latitude for each region.

3.8 Tables

Table 1 – Samples and population designations

Mountain region, population identifier for Figure 1, population name, total number of individuals (n_{tot}) and number of those samples from "antique" DNA (n_a).

Mountain Region	**Ident.**	**Population**	n_{tot}	n_a
Alaska Range	a.	Southwest Alaska Range	17	1
	b.	Denali	5	
	c.	Central Alaska Range	23	
Chugach Mountains	d.	Anchorage	10	
	e.	Thompson Pass	1	
	f.	Eastern Chugach	13	
Yukon-Tanana Uplands	g.	White Mountains	12	
	h.	Yukon-Charley NPP	38	
	i.	Ogilvie Mountains	7	7
Wrangell-St. Elias	j.	Wrangell Mountains	10	1
	k.	Kluane	14	
Coast Mountains	l.	Whitehorse	2	1
	m.	British Columbia	7	5
	n.	Canol Road	1	1
Mackenzie Mountains	o.	Northwest Territories	4	2
	p.	Bonnet Plume Lake	1	1
	q.	Keele Lake	2	2
	r.	Ross River	1	1
	s.	Little Hyland River	1	1
Richardson Mountains	t.	Horn Lake	4	4

Table 2 – Primers

Primers used in the amplification sequencing of fresh and antique tissues

Fragment length*	**Primer name**	**Sequence (5' to 3')**
238 bp	CB-HLF1**	CCACCGTTGTAGTTCAACTA
	CB-HLaR1	TGCGCCGTTAGCGTGCAGGTAG
271 bp	CB-HLaF2	GACCTCCCAACCCCCTCAAACATCTC
	CB-HLaR2	CGGTAGCTATGACTGCGAATAATA
269 bp	CB-HLaF3	ACATCAGACACACTAACAGCATT
	CB-HLaR3	CAAGGTCGGTGCCGATGTACGGGA
248 bp	CB-HLaF4	ACACCTACTCAGAAACATGGAA
	CB-HLiR2**	AGCCTGTTTCGTGGAGGAAGAGTA
269 bp	CB-HLaF5	CCTTCTGAGGGGCAACCGTAA
	CB-HLaR5	CTAGGGTGAGGAGGAGGGAGAT
253 bp	CB-HLiF2**	AGCCACCCTAACTCGATTCT
	CB-HLaR6	GGTTTGATATGTGGAGGTGTG
272 bp	CB-HLaF7	CAGGAATCATCCCAGACGCAGAC
	CB-HLaR7	TGCTTCGTTGTTTTGATGTGTG
215 bp	CB-HLaF8	GAGACCCAGACAACTACACCC
	CB-HLaR8	CATGTGAGTGTGAGTAGATCGGC
256 bp	CB-HLaF9	ACTTGGCGGCGTATTAGCCCTTAT
	CB-HLR1**	GGTTTACAAGACCAGGGTA

*Not including primers

** Previously published in Lanier & Olson (2009)

Table 3 – Diversity statistics

Population genetic summary statistics computed in DnaSP (Librado and Rozas 2009): *n* = number of samples, S=number of segregating sites, *h* = number of haplotypes, *Hd* = haplotype diversity, π = per site nuclear diversity, *k* = mean number of pairwise nuclear differences, Ramos-Osins and Rozas's R_2, Fu's *Fs*, and Fay and Wu's *H*. Diversity statistics are not shown separately for populations with < 4 samples, but those samples were included in regional comparisons. Test statistics significant at the p < 0.05 level (tested with 10,000 coalescent simulations), in bold.

	Population	n	*S*	*h*	H_d	π	*k*	R_2	*Fs*	*H*
Alaska Range		45	29	16	0.91	0.0046	5.170	0.085	-2.097	1.666
a.	SW Alaska Range	17	6	5	0.772	0.002	1.779	0.144	0.114	-1.191
b.	Denali	5	11	5	1	0.0052	5.676	0.203	-1.113	0.4
c.	Central Alaska Range	23	13	6	0.771	0.003	3.905	0.148	2.131	1.265
Chugach Mountains		24	24	9	0.859	0.0065	7.225	0.146	1.889	3.318
d.	Anchorage	10	13	6	0.889	0.005	5.333	0.188	0.541	1.778
f.	Eastern Chugach	13	13	3	0.653	0.006	6.692	0.257	**7.499**	-0.115
Yukon-Tanana Uplands		57	32	22	0.936	0.0054	6.053	0.091	-4.382	2.847
g.	White Mountains	12	2	2	0.53	0.001	1.061	0.265	2.535	-0.424
h.	Yukon-Charley NPP	38	28	15	0.902	0.005	5.677	0.096	-1.609	-4
i.	Ogilvie Mountains	7	6	5	0.905	0.0028	3.143	0.245	-0.612	0.952
Wrangell-St. Elias		24	31	15	0.942	0.0067	7.471	0.111	-2.656	-1.355
j.	Wrangell Mountains	10	24	6	0.778	0.006	6.778	0.127	1.121	2.222
k.	Kluane	14	10	9	0.934	0.003	3.474	0.156	-2.981	1.013
Coast Mountains		10	12	7	0.933	0.0037	4.156	0.175	-1.19	1.422
m	British Columbia	7	12	5	0.905	0.0039	4.381	0.18	0.039	2.429
Mackenzie Mountains		9	27	9	1	0.0058	6.500	**0.073**	**-4.01**	**5.5**
o.	Northwest Territories	4	17	4	1	0.009	8.833	**0.12**	0.188	**5**
Richardson Mountains										
t.	Horn Lake	4	8	3	0.833	0.0037	4.167	0.227	1.225	-0.333
	All *O. collaris*	173	115	79	0.983	0.007	8.374	**0.035**	**-58.54**	

Table 4 – Population pairwise Φst measurements

Most populations in our study are significantly differentiated based on pairwise Φst statistics. Bold values are significant at the 0.05 level based on 1000 permutations.

	Pop	a	b	c	d	f	g	h	i	j	k	m	o
a	SW AK Range	-											
b	Denali	**0.58**	-										
c	Central AK Range	**0.53**	**0.34**	-									
d	Anchorage	**0.54**	**0.29**	**0.31**	-								
f	E. Chugach	**0.55**	**0.31**	**0.41**	**0.30**	-							
g	White Mtns	**0.84**	**0.75**	**0.69**	**0.70**	**0.62**	-						
h	Yukon-Charley	**0.43**	**0.26**	**0.32**	**0.33**	**0.33**	**0.42**	-					
i	Ogilive Mtns	**0.59**	**0.28**	**0.28**	**0.29**	**0.35**	**0.78**	**0.27**	-				
j	Wrangell Mtns	**0.53**	**0.26**	**0.38**	**0.32**	**0.19**	**0.60**	**0.21**	**0.30**	-			
k	Kluane	**0.81**	**0.68**	**0.71**	**0.69**	**0.43**	**0.82**	**0.60**	**0.73**	**0.53**	-		
m	British Columbia	**0.80**	**0.63**	**0.67**	**0.64**	**0.35**	**0.84**	**0.57**	**0.70**	**0.47**	**0.36**	-	
o	Northwest Terr.	**0.50**	0.09	**0.29**	**0.23**	**0.29**	**0.71**	**0.24**	0.13	**0.22**	**0.67**	**0.59**	-
t	Richardson Mtns	**0.60**	0.19	**0.30**	**0.26**	**0.29**	**0.76**	**0.22**	**0.26**	0.13	**0.70**	**0.66**	0.05

Table 5 – AMOVA results

Analysis of molecular variance (AMOVA) comparing genetic variance with mountain ranges, within populations within ranges, and within populations.

Source of variation	**Variance component**	**% of variation**	**p-value**
Among mountain ranges	0.845	18.53	<0.0001
Among populations within ranges	1.491	32.69	<0.0001
Within populations	2.225	48.78	0.0078

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3.10 Appendix – Voucher specimens & geography

Mountain range, locality ID, population, and museum catalog number for samples used in this study. Starred samples (*) from antique sources. CMN= Canadian Museum of Nature, Hik = samples from David Hik (University of Alberta Edmonton), KU = University of Kansas Natural History Museum & Biodiversity Research Center, UAM = University of Alaska Museum, USNM = US National Museum

Mountain Range	ID	Population	Institution & Catalog Number
Alaska Range	a	Southwest Alaska Range	UAM 100773; UAM 100775; UAM 100776; UAM 100795; UAM 100796; UAM 100833; UAM 100834; UAM 100839; UAM 100840; UAM 100848; UAM 100849; UAM 100867; UAM 100870; UAM 100890; UAM 100943; UAM 93191; USNM 13651*
	b.	Denali	UAM 98296; UAM 102573; UAM 102574; UAM AF64794; UAM 102561
	c.	Central Alaska Range	UAM 102478; UAM 102481; UAM 102482; UAM 102483; UAM 102486; UAM 102487; UAM 102488; UAM 102490; UAM 102492; UAM 10249; UAM 102494; UAM 102495; UAM 102496; UAM 102497; UAM 102498; UAM 102500; UAM 102501; UAM 102502; UAM 102503; UAM 102504; UAM 102505; UAM 102506; UAM 102507
Chugach Mountains	d.	Anchorage	UAM 31645; UAM 31646; UAM 31647; UAM 51299; UAM 64363; UAM 102564; UAM 102565; UAM 102566; UAM 102567; UAM 102568
	e.	Thompson Pass	UAM 100896

	f.	Southern Wrangell-St. Elias	UAM 57694; UAM 102419; UAM 102420; UAM 102422; UAM 102423; UAM 102424; UAM 102429; UAM 102430; UAM 102431; UAM 102432; UAM 102434; UAM 102435; UAM 102438
Yukon-Tanana Uplands	g.	White Mountains	UAM 63931; UAM 63932; UAM 63935; UAM 63936; UAM 63938; UAM 67030; UAM 102554; UAM 102556; UAM 102557; UAM 102558; UAM 102559; UAM 102560
	h.	Yukon-Charley Rivers NPP *	UAM 58204; UAM 58205; UAM 58206; UAM 58207; UAM 58208; UAM 58211; UAM 58212; UAM 58213; UAM 58214; UAM 58219; UAM 58242; UAM 58243; UAM 58244; UAM 58245; UAM 58248; UAM 58249; UAM 58250; UAM 58251; UAM 58257; UAM 58258;UAM 58316; UAM 58338; UAM 58399; UAM 58400; UAM 58407; UAM 58422; UAM 58444; UAM 58445; UAM 58446; UAM 58448; UAM 58546; UAM 58547; UAM 58548; UAM 58549; UAM 58550; UAM 58551; UAM 58552; UAM 58553
	i.	Ogilvie Mountains	CMN 29410*; CMN 29411*; CMN 30647*; CMN 44998*; CMN 45001*; CMN 45002*; CMN 45006*
Wrangell-St. Elias	j.	Wrangell Mountains	KU 147383*; UAM 102366; UAM 102416; UAM 56066; UAM 56067; UAM 56093; UAM 56094; UAM 56099; UAM 56814; UAM 57920
	k.	Kluane	Hik 1205; Hik 1207; Hik 1326; Hik 1329; Hik 1355; Hik 1385; Hik 1555; Hik 1628; Hik 1649; Hik 431; Hik 441; Hik 446; Hik 492; Hik 499
Coast Mountains	l.	Whitehorse	CMN 31161*; UAM 71652

	m.	British Columbia	KU 29099*; KU 29101*; UAM 35126; UAM 51896; USNM 127142*; USNM 128581*; USNM 128582*
	n.	Canol Road	CMN 30648*
Mackenzie Mountains	o.	Northwest Territories	CMN 18106*; CMN 42788*; UAM 88532; UAM 88534
	p.	Bonnet Plume Lake	CMN 35314
	q.	Keele Lake	CMN 35320*; CMN 35328*
	r.	Ross River	CMN 17803*
	s.	Little Hyland River	CMN 31167*
Richardson Mountains	t.	Horn Lake	CMN 30301*; CMN 30302*; CMN 30304*; CMN 46541*

* (shown as Yukon-Charley NPP in figures and tables)

Chapter 4:

Diversification, demography and descent: Comparative phylogeography of five alpine mammals in eastern Beringia[1]

4.1 Abstract

Studies have shown that alpine and arctic organisms may be more likely to exhibit recent range contraction into interglacial refugia as opposed to post-glacial expansion out of refugia. Extant patterns of genetic diversity among different codistributed species likewise may be indicative of a common history of vicariance or idiosyncratic responses to Quaternary climate change. We use a combination of phylogeographic inference, Bayesian skyline plots, and hierarchical Approximate Bayesian Computation to test for phylodemographic concordance within five species of alpine-adapted small mammals in eastern Beringia. These five species (collared pikas, hoary marmots, singing voles, brown lemmings, and arctic ground squirrels) vary in their degree of specificity to alpine and boreal-tundra habitat but exhibit sufficient commonalities (e.g., cold tolerance and nunatak survival) that we might expect them to share concordant phylogeographic and demographic patterns. Divergence levels did not support recent post-Pleistocene expansion from sub-Laurentide populations in any of the species we compared. Because there was strong support for a single simultaneous divergence event, it is likely that intra-Beringian divergences occurred in all five species, although local differences in abundance are likely. The Wrangell-St. Elias region was likely an important zone of

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[1] Lanier, H. C., A. M. Gunderson, M. Weksler, V. B. Fedorov, and L. E. Olson. 2010. Diversification, demography and descent: Comparative phylogeography of five alpine mammals in eastern Beringia. Prepared for submission to Evolution.

recent secondary contact for divergent lineages of alpine small mammals, and the genetic diversity we observed in this area was higher than for any other area of our study. Bayesian skyline plots for four of the five species in our study do not support a history of Pleistocene population contraction. Brown lemmings showed evidence of recent demographic expansion without subsequent population decline. Finally, the two most divergent and genetically diverse species were brown lemmings and singing voles. These species undergo higher amplitude population fluctuations and exhibit higher effective population sizes than the other species we compared. Higher effective population size, shorter generation time, higher litter size, and greater per-female reproductive output, may yield evolutionary advantages as these species adapt to climate change.

4.2 Introduction

Quaternary climate changes have strongly structured the spatial distribution of genetic variation in many species (Hewitt 2004). Drastic post-Pleistocene climate change resulted in demographic expansion into previously glaciated areas for many species (Lessa et al. 2003). Phylogeographic studies suggesting Pleistocene contraction into refugia followed by post-Pleistocene expansion abound (Byun et al. 1997; Branco et al. 2002; Brunhoff et al. 2003; Alsos et al. 2005; Leonard et al. 2005; Eidesen et al. 2007; Aubry et al. 2009; Stewart et al. 2010). For northern-distributed taxa in North America, one of the most influential refugia was Beringia, the vast ice-free corridor formerly connecting North America and Asia (Hultén 1937; Hopkins 1967). During the Pliocene and Pleistocene, Beringia functioned as a land bridge allowing biotic interchange between Asia and North America (Simpson 1940), as a barrier to dispersal (Hopkins et al. 1982), and as a center of endemism structuring the diversity of the species in the region (Sher 1999; Cook et al. 2005; Eidesen et al. 2007; Waltari et al. 2007b).

During previous glacial cycles, the Laurentide and Cordilleran ice sheets blanketed much of North America and isolated plant and animal species in multiple refugia. Although Pleistocene speciation models have been rejected for a number of North American taxa (Near et al. 2003; Lanier and Olson 2009), glacial separation and subsequent unification of taxa has resulted in introgression, competition, and/or replacement between divergent lineages within species (Macpherson 1985; Ibrahim et al. 1996; Hewitt 2000; Stewart et al. 2010). Cycles of repeated isolation, localized extirpation, and ensuing recolonization depressed genetic diversity in many northern species relative to their southern relatives (Hewitt 2000; Lessa et al. 2003). In addition to the Beringian refugium, cryptic northern refugia appear to have played an important role in the recolonization of previously glaciated regions (Macpherson 1985; Fedorov and Stenseth 2002). Other unglaciated areas including "easternmost Beringia" (Fig. 1f), an area in the Mackenzie Mountains of the

Northwest Territories (Duk-Rodkin and Hughes 1991), and regions between adjacent ice sheets in northern British Columbia (Catto et al. 1996) may also have served as refugia. Dominated by herbs and shrubs during previous glaciations (Szeicz and MacDonald 2001), these regions are thought to have maintained populations of Dall sheep and alpine plants (Loehr et al. 2006; Marr et al. 2008). Nunataks, emergent rock "islands" surrounded by glaciers and supporting plant and animal life, also may have served as cryptic refugia preserving populations and genetic diversity for some cold-tolerant species (Murray and Murray 1969; Youngman 1975; Holderegger and Thiel-Egenter 2009).

4.2.1 Beringian phylogeographic patterns

Genetic, geological, and parasitological evidence suggests that the majority of faunal interchange across Beringia occurred from west to east, or from Asia to North America (Simpson 1940; Waltari et al. 2007b). Phylogeographic-level studies on shorter timescales run contrary to this pattern, with the majority of the recently colonizing species in eastern Beringia (present-day Alaska) arriving via North American routes (Weksler et al. accepted). To date, six major phylogeographic patterns have been described in the mammals of eastern Beringia (Figure 1; reviewed by Weksler et al. accepted): a) recent colonization from sub-Laurentide populations via a single route, b) recent colonization from sub-Laurentide populations via separate continental and coastal routes, c) recent colonization of Beringia from east Asia via the Bering Land Bridge, d) vicariance between populations in Beringian and sub-Laurentide refugia, e) vicariance between populations in Beringian and Canadian high-Arctic refugia, and f) intra-Beringian diversification. Cold-adapted species show more genetic variation and are more likely to stem from Beringian populations, whereas boreal and temperate forest taxa appear to be recent colonizers (Youngman 1975; Macpherson 1985; Cook et al. 2005; Weksler et al. accepted).

For several species that underwent vicariance between Beringian and sub-Laurentide or high-Arctic populations, secondary contact at approximately the Alaska-Canada border has been suggested (Fedorov and Goropashnaya 1999; Fedorov and Stenseth 2002; Fleming and Cook 2002; Fedorov et al. 2003). Fedorov et al. (2003) found that brown lemmings (*Lemmus trimucronatus*) fell into two major phylogeographical groups: a well-supported Beringian clade (hereafter W) currently found in Alaska and Russia and a Mackenzie drainage clade to the east (hereafter E). Following Macpherson (1985), Fedorov et al. (2003) concluded that the eastern (E) lineage was previously isolated in sub-Laurentide areas. Phylogeographic inferences for the singing vole (*Microtus miurus*) were more ambiguous (Weksler et al. accepted). Singing voles show evidence of several successive population fluctuations within Beringia, as well as divergence of that group from the easternmost samples from the Wrangell Mountains. With only two individuals from the eastern lineage they were unable to distinguish between hypothesized intra-Beringian divergence (pattern f) and a history of sub-Laurentide vicariance with subsequent recolonization (pattern d). Phylogeographic studies of a third co-occurring alpine and arctic tundra specialist, the arctic ground squirrel (*Spermophilus parryii*), found three main lineages that may have diversified in Beringia (Eddingsaas et al. 2004). One of these lineages occurs north of the others in the Brooks Range, one is found in the southwest along the Alaskan Peninsula, and one is more easterly distributed but also occurs on the Seward Peninsula and St. Lawrence Islands. This pattern has been interpreted to be evidence of a long history of diversification within the Beringian refugium (pattern f; Eddingsaas et al. 2004; Weksler et al. accepted).

4.2.2 Alaskan alpine mammals

Recent research indicates that the Pleistocene contraction/post-Pleistocene range expansion model commonly applied to temperate species may not be appropriate for cold- and alpine- adapted species (Dalén et al. 2006; Provan and

Bennett 2008; Galbreath et al. 2009; Stewart et al. 2010). Although extensive glaciations in the north (Figure 2f) may have greatly limited the available habitat, recent studies of debris-covered glaciers and nunataks have uncovered a surprising diversity of species that can persist in these habitats (Fickert et al. 2007). Several authors have recently suggested that the term "refugium" should be applied to the area of maximum range contraction for a species (Stewart et al. 2010). Thus, although the five primary species of alpine-associated small mammals found in eastern Beringia vary in their degree of affiliation with alpine and boreal-tundra habitat, we might expect them to demonstrate concordant phylogeographic and demographic patterns shaped by common historical events (Table 1; Figure 2). First, they occur and thrive above treeline (Tyser 1980; Gillis et al. 2005) and are present along glacial margins as well as on nunataks (Murray and Murray 1969; MacDonald and Jones 1987). Second, they were present and (in the case of singing voles and arctic ground squirrels) common and widespread in eastern Beringia during the Pleistocene (Kurtén and Anderson 1980; MacDonald and Cook 2009).

Although there are differences in their phylogeographic histories, fossil evidence suggests a Pleistocene presence in Beringia for all of the five focal species in our study (Table 1; Harington 2003). Four of the species we are studying—singing voles, brown lemmings, arctic ground squirrels, and collared pikas (*Ochotona collaris*)—are well known from fossil localities in Alaska. Fossil evidence is ambiguous for hoary marmots (*Marmota caligata*), which are difficult to distinguish morphologically from the Alaskan marmot (*M. broweri*; Gunderson et al. 2009). Hoary marmots and collared pikas are the more narrowly distributed species (MacDonald and Cook 2009) and provide an important contrast in terms of relative demography and dispersal ability. In terms of timing we might expect that species that diversified within Beringia (Figure 1f) diverged simultaneously or nearly simultaneously with one another but asynchronously compared to species that experienced vicariance between Beringian and sub-Laurentide populations (Figure 1d). Our dataset affords an opportunity to test for guild-wide responses to

past climate change and to look at whether subarctic alpine small mammals responded in a uniform fashion to post-Pleistocene fluctuations. This in turn may enable subsequent predictions regarding ongoing and future responses to climate change.

4.2.3 Objectives

We seek to compare phylogeographic patterns for five species of alpine-adapted eastern Beringian mammals to look for commonalities in pattern and inferred historical processes. Although a number of these species have been characterized separately, applying a model-based comparative phylogeographic approach allows us to test for the relative influence of shared historical events and concerted evolutionary changes (Hickerson et al. 2010). Specifically, we tested whether codistributed alpine small mammals responded to Quaternary climate change uniformly and whether diversity patterns are indicative of a common history of inter-Beringian diversification, vicariance between Beringian and sub-Laurentide populations, or if there are idiosyncratic patterns that differ between species (see patterns and expectations in Table 2). We also used a coalescent model to determine if these cold-adapted species show evidence of range contraction into interglacial refugia as opposed to post-glacial expansion out of refugia.

4.3 Methods

4.3.1 Phylogeographic analyses

We assembled a dataset of mitochondrial cytochrome *b* sequences for five co-distributed small mammal species from alpine areas of eastern Beringia. For published studies (Fedorov et al. 2003; Eddingsaas et al. 2004; Weksler et al. accepted), data were retrieved from GenBank. Cytochrome *b* sequences for hoary marmots and collared pikas were generated as described previously (Gunderson 2007; Lanier and Olson 2009). In total, we included 77 collared pikas (representing 17 unique localities), 26 hoary marmots (15), 37 singing voles (19), 28 brown

lemmings (14), and 48 arctic ground squirrels (19) in the comparative phylogeographic dataset. Geographic distributions, phylogeographic subdivisions, and sample sizes are shown in Figure 2 and Table 3. Most of the samples are associated with specimens in the University of Alaska Museum's mammal collection(Appendix A).

Initially, we used phylogenetic trees and sampling maps to compare phylogeographic distributions. We used jModelTest (Guindon and Gascuel 2003; Posada 2008) to compare 88 possible nucleotide substitution models on a maximum likelihood (ML) optimized tree. We used the Akaike Information Criterion corrected for small sample sizes (AICc) to select between models (Posada and Buckley 2004). While the small sample size correction may not be necessary for this dataset, AICc converges with AIC as sample size increases (Burnham and Anderson 2003). Heuristic tree searches and bootstrapping under the ML criterion were conducted using GARLI (v. 0.942; Zwickl 2006). Maximum likelihood trees and models of evolution are given in Appendix B. We classified west (W; central Beringian) and east (E; east of central Beringia) lineages as those clades separated by the longest internode in each unrooted ML tree. These lineages correspond to mostly western and mostly eastern geographic distributions, respectively. The northern lineage (N; Brooks Range, Alaska) in arctic ground squirrels was treated separately.

Population genetic summary statistics (Table 3) were computed in DnaSP v. 5 (Librado and Rozas 2009). To compare genetic diversity within clades, we computed the number of haplotypes (h), the number of segregating sites (S), and the per-site effective population size parameter (θ; the product of effective population size and mutation rate) based on π under a finite sites model (Tajima 1996). To compare within-species divergences, we calculated the net number of nucleotide substitutions between E and W clades (Da; Nei 1987). We used several different summary statistics to look for evidence of demographic expansion: Tajima's D, Fay and Wu's H, and Fu's Fs. A standard statistical test used to identify both selection and demographic history, Tajima's D (Tajima 1989) compares θ estimated from π

with 0 estimated using segregating sites to look for departures from neutrality. Tajima's *D* is particularly sensitive to demographic expansion (Zeng et al. 2006). Positive values are considered as evidence of a recent population bottleneck whereas negative values are indicative of population expansion. We also took a second approach, Fay and Wu's *H*, which may be more sensitive than other metrics at small sample sizes (Fay and Wu 2000; Carnaval et al. 2009). Fay and Wu's *H* contrasts low- and intermediate-frequency alleles and is more sensitive to population decline and population subdivision than Tajima's *D* (Zeng et al. 2006). We contrast these two methods based on 0 estimators with Fu's *Fs*, a method based on the haplotype frequency distribution that may be more sensitive than Tajima's *D* to population expansion (Fu 1997). Significance for each of these tests was calculated at a 95% level using 10,000 coalescent simulations in DnaSP.

4.3.2 Demographic analyses

While summary statistic approaches can be useful, coalescent-based approaches take full advantage of the genealogical relationships of the alleles (Felsenstein 1992; Kuhner et al. 1995). To compare and contrast demographic histories within and between the alpine small mammals of eastern Beringia, we used a series of Bayesian skyline plots (BSPs) implemented in BEAST 1.4.8 (Drummond and Rambaut 2007). This approach employs a flexible coalescent prior to examine fluctuations in demographic trends over time. For each species, we used the model of evolution determined by ModelTest. We performed three runs (for 10 million, 20 million, and 50 million generations, respectively) to check for convergence. Trees and parameters were sampled every 1000 generations, and the initial 10% of the output was removed as burn-in. We used Tracer v1.5 to verify effective sample size and construct BSP intervals.

4.3.3 Concordance testing

We used hierarchical Approximate Bayesian Computation (hABC) implemented in msBayes (Hickerson et al. 2006b) to evaluate the number of simultaneous divergence events occurring within our five focal taxa. This flexible, simulation-based approach can account for differences in mutational variance, coalescent variance, and demographic history to determine the probable number of synchronous divergences among a set of species pairs (Hickerson et al. 2006a; Hickerson et al. 2007). We performed two separate analyses in msBayes. The first included up to 20 (in most cases, all) individuals from each of the clades (E-W split) of all five species with the northern lineage of arctic ground squirrels excluded. The second analysis was the same for all taxa except arctic ground squirrels. Because arctic ground squirrels exhibited two exceptionally long internodes, we conducted a series of separate analyses using the northern clade (N) and a combined E-W ($S_{E\text{-}W}$) subsample as sister groups. Because of the simulation approach implemented in msBayes, datasets with small samples can produce robust results (Hickerson et al. 2007). As predicted under coalescent theory, datasets larger than 20 individuals per species pair increased the computation time and the variance without greatly influencing the results. For both analyses we set upper and lower bounds on the prior for theta ($0 < \theta < 100$), an upper bound on the time of separation tau ($\tau < 40$), a uniform prior on the number of tau classes with different values, and the default prior on the upper limit for the ancestral population size. We conducted 500,000 replicates for each analysis and used a tolerance of 0.001 to yield approximately 500 draws from which to construct posterior intervals.

4.4 Results

4.4.1 Pattern comparison

From a qualitative perspective, the Beringian (W) and eastern (E) phylogroups described in Fedorov et al. (2003) and suggested by Weksler et al.

(accepted) were present in all five species (Figures 2 and 3). Relatively deep subdivision was apparent between phylogroups for brown lemmings and singing voles. Divergence between clades was less (2.5%) for hoary marmots and much less (0.6%) for collared pikas (Figure 3). Arctic ground squirrels include two clades south of the Brooks Range, one with a more "eastern" distribution but with some longitudinal overlap on islands around Alaska (Figures 2d and 3d). As mentioned previously, there is a northern lineage of arctic ground squirrels that is sister to the E-W lineage (Figure 2e). Geographically, the contact zones between clades varied widely (Figure 2). Contact zones within brown lemmings, collared pikas, and singing voles might be considered to be roughly coincident with Wrangell-St. Elias National Park and Preserve (NPP) and the Alaska-Canada border. The contact zone within hoary marmots was further to the east, and both lineages were present in a population in northern British Columbia. Arctic ground squirrels contain regional phylogeographic differentiation, but traditional phylogeographic assessment did not indicate a strong degree of concordance with the other four species.

The greatest degree of genetic divergence between clades (Figure 3) and genetic diversity within each (W or E) clade was found in brown lemmings and singing voles (Table 3). Values of θ within both brown lemming and the western singing vole phylogroups were approximately twice those in collared pikas and arctic ground squirrels and ten times those in hoary marmots. The lowest values for the diversity metrics were recovered in hoary marmots. None of the species showed evidence of eastern population expansion and western demographic stability. On the contrary, only the western lineages of collared pikas and singing voles and both lineages of brown lemmings showed significant expansion signals (based on significant values for Fu's *Fs*). Tajima's *D* (which is considered to be less sensitive than Fu's Fs; Fu 1997) indicated a significant population expansion in only the western clade of brown lemmings. Fay and Wu's *H* was significant only for the western clade of singing voles.

4.4.2 Demographic histories

Bayesian skyline plots (Figure 4) recovered a greater variety of demographic signals than did summary statistics. Pleistocene range expansion followed by recent demographic contractions appears to have occurred in singing voles, collared pikas and possibly Arctic ground squirrels. Contrary to our expectations, the BSP analysis indicated a strong signal of Pleistocene and recent demographic expansion as well as a higher (1-2 orders of magnitude) long-term effective population size for brown lemmings. There was a hint of recent demographic decline in hoary marmots, but no strongly supported trend in either direction. The exceptionally low within-clade diversity in hoary marmots may make it difficult for coalescent methods to distinguish between alternate demographic histories with any confidence.

4.4.3 Concordance testing

Using the hABC approach, strong support was recovered for a history of simultaneous vicariance. Despite the differences in clade depth and distribution, there was strong support for simultaneous divergence between the eastern and western clades for all five species. The mode of the distribution of independent divergence events (Ψ_{mode}) was 1.154 (95% HPD interval: 1.00-1.91). When divergence was simultaneous, the ratio of variance to expected divergence time (Ω = Var[t]/E[t]) should approach zero, and for this dataset the mode for the distribution was essentially zero (Ω_{mean} =0.007, 95% HPD interval: 0.00-0.03). When we used the N- S_{E+W} split in arctic ground squirrels instead of the E-W split, there was also support for simultaneous divergence (Ψ_{mode} = 1.231; 95% HPD interval: 1.00-2.09; Ω_{mean} = 0.10; 95% HPD interval: 0.00-0.20). While the modes of these distributions are similar, the dataset with the W_N - E_{E-W} split in arctic ground squirrels exhibited a slightly longer tail encompassing greater variation in the range of Ψ, Ω, and the expected time to divergence.

4.5 Discussion

4.5.1 Biogeographic history of eastern Beringia

Our results suggest simultaneous vicariance between populations of all five species in central Beringia (W) and eastern regions (E). These eastern regions could have included some combination of ice sheet margins, nunataks, and various ice-free areas between the Laurentide and Cordilleran ice sheets. None of the eastern lineages exhibited a greater signal of population expansion than the western lineages for any of the species in our study. None of the eastern lineages exhibited greatly reduced genetic diversity (with the exception of singing voles, for which eastern sampling was limited to two individuals). Because there was strong support for a single simultaneous divergence event, it seems likely that Pleistocene geographic distributions were similar for all five species, although differences in local abundance and ancestral population size are likely. Pleistocene population centers within eastern Beringia (currently central Alaska) and easternmost Beringia likely differed at fine scales by species and over time, but produced broadly similar phylogeographic patterns for the five species we compared. The contrast between Fay and Wu's *H* and Tajima's *D* is indicative of greater population subdivision for singing voles than for the other species in our study (Zeng et al. 2006). Nunataks and cryptic refugia, such as easternmost Beringia (Szeicz and MacDonald 2001), shaped the phylogeographic patterns in the alpine mammals of eastern Beringia. However, based on median demographic trends (Figure 4), it may be misleading to term these areas "refugia" for most of our study species as they seem unlikely to represent demographic and geographic minima (Stewart et al. 2010).

4.5.2 Estimating simultaneous divergences

We were surprised to find that simultaneous divergence in our dataset was equally well supported in comparisons whether we used the E-W split or the N-S_{E+W} split for arctic ground squirrels. While all of these splits represent recent

divergences, hABC techniques are able to distinguish between non-simultaneous divergent events in other recently diverged northern sister species and intraspecific comparisons (Humphries 2008; Topp and Winker 2008). Divergences between all three arctic ground squirrel clades (E, W, and N) may be sufficiently simultaneous that there is no temporal difference recovered in our analysis. Within and between-group diversity metrics for arctic ground squirrels do not differ from those of the other species under a model of simultaneous divergence.

4.5.3 Contact zone dynamics in Wrangell-St. Elias

While ancestral areas tend to harbor older genotypes, the regions with the greatest genetic diversity tend to be suture zones between disparate groups (Petit et al. 2003). The Wrangell-St. Elias region appears to be an important contact zone for alpine mammals, and the genetic diversity in this area was higher than in any other area of our study. The combined protected areas of Wrangell-St. Elias NPP and the neighboring Kluane NPP in the Yukon Territory constitute a considerable segment of the second-largest protected area in the world (Danby and Slocombe 2005). We know relatively little about the mammals of this region, with several new species records revealed by recent inventory work (Cook and MacDonald 2003). In-depth ecological and evolutionary studies of Wrangell-St. Elias mammals might also reveal more about the role that glacial margins and nunataks play in preserving and structuring diversity of alpine organisms and the colonization of previously glaciated regions by these species. Additional questions regarding contact zone dynamics and directionality of colonization at different divergence levels could be addressed in the region. The high genetic divergence within singing voles and brown lemmings may be result in competition between divergent phylogroups where secondary contact occurs (Hewitt 2001). Collared pikas and hoary marmots exhibit lower between-clade divergences. Co-occurrence of both haplotype groups in the same populations leads us to believe that competition and adaptive divergence are unlikely. From an ecological, evolutionary, and climate change

perspective, this region has much to offer in terms of future studies of divergence and introgression in alpine organisms.

4.5.4 "Interglacial refugia" or cryptic refugia?

> "Quaternary refugia should be defined as the geographical region or regions that a species inhabits during the period of a glacial/interglacial cycle that represents the species' maximum contraction in geographical range" – (Stewart et al. 2010)

The cold, windswept "mammoth-steppe" of central Beringia during the Pleistocene is thought to have been similar to communities of plants and animals currently found in the alpine and boreal tundra of Alaska and northern Canada (Kurtén and Anderson 1980; Guthrie 1982; Zazula et al. 2006). Species of plants and animals that are now confined to disjunct alpine habitats appear to have been widespread at lower elevations during the Pleistocene (Guthrie 1982). Bayesian skyline plots for four of the five species in our study do not support a history of Pleistocene population contraction, and terming the ancestral locations for the recovered phylogroups as "refugia" may not be appropriate.

When contrasting putative areas of "maximum contraction" (Stewart et al. 2010), both geographic and demographic factors are important. The difference between Pleistocene and current ranges is influenced by the quality of our knowledge of ranges at both time periods. Estimates of habitat availability based on the extent of glaciation at the Last Glacial Maximum (LGM) alone (e.g., Waltari et al. 2007a; Figure 2f) probably underestimate historic ranges of our focal species for several reasons. First, the Pleistocene presence of glacial margin (peripheral; sensu Holderegger and Thiel-Egenter 2009) and nunatak populations are likely to have increased the available habitat for species. Second, the LGM was a relatively short period (Clark et al. 2009) when compared to the length of the expansion and contraction phases of glacial cycling. Suitable climate for alpine-adapted animals was more extensive during and immediately following the LGM (Pielou 1991) and

has subsequently been reduced. Poor resolution of species margins on range maps can also lead to errors of commission (false occurrences; Gaston and Fuller 2009) when considering the current distribution and abundance of northern species. The range maps we present (Figure 2) represent a composite of commonly published maximum extent and not necessarily accurate assessments of suitable habitat. We believe this to be a continuing challenge in the study of Alaskan mammals; distribution maps for species in northern regions are often less accurate than those for their southern congeners. For the Alaska marmot (*M. broweri*) and the hoary marmot (*M. caligata*), most published and freely available range maps include the range of the former with the latter, although the two are not known to occur in sympatry (Gunderson et al. 2009). Many range maps are based on museum specimen records, and temperate North America has seen a longer and more extensive history of museum collecting than the Nearctic. In terms of collected museum specimens, research on collared pikas is approximately 100 years behind the American pika (*O. princeps*; Lanier *in prep*). Seemingly broad published distribution maps that do not account for discontinuities in occurrence can artificially inflate the extent of occurrence for a species (Gaston and Fuller 2009).

Instead of considering the proposed regions of origin for each lineage (E or W) refugia, it might be more accurate to refer to them as Pleistocene population centers. For species that have experienced Pleistocene population expansion and post-Pleistocene contraction, it might be more appropriate to term their reduced areas of occupation "interglacial refugia" (Stewart et al. 2010). While this may seem like a minor distinction, there may be heuristic value in the recognition that recent climatic trends are stressing species whose distributions and abundances may already be declining. Recent range reduction and population extirpation noted in species such as the Olympic marmot (Griffin et al. 2008), American pikas (Beever et al. 2003), and the Alaska marmot, *M. broweri* (Gunderson 2007), may presage decline for other alpine and arctic adapted species (Krajick 2004; Smith et al. 2004).

Unlike demographic trajectories inferred for the other four species, there is evidence of recent demographic expansion without subsequent population decline in brown lemmings. While there is a moderate Beringian fossil record for brown lemmings (Repenning et al. 1964; Guthrie and Matthews 1973; Jopling et al. 1981), this does not preclude the possibility that they underwent range decreases and population reduction during the last glacial maximum. Post-Pleistocene changes in available habitat may have mediated demographic shifts within brown lemmings. Brown lemmings appear to prefer mesic habitat (Batzli and Lesieutre 1995), which has increased since the end of the Pleistocene (Guthrie 2003). We can't reject the refugial contraction/post-Pleistocene expansion model for brown lemmings. It remains to be seen whether this post-Pleistocene expansion may be checked by recent global warming trends.

4.5.5 Effective population size and population cycles

Differences in the intraspecific divergence shown by the taxa in our study highlight the role that fecundity, dispersal capability, and high-amplitude population fluctuation may play in preserving genetic diversity and increasing effective population size (Erhich and Jorde 2005). The two most genetically diverse and intraspecifically divergent species (brown lemmings and singing voles) undergo higher amplitude population fluctuations and exhibit higher effective population sizes (Figure 3, Table 4). Although the low-cycles during population fluctuation might be expected to decrease effective population size in these species, they still exhibit higher effective sizes relative to the lower-density, lower-amplitude hoary marmots, collared pikas, and arctic ground squirrels. The multigenerational effective size of these species is calculated as the harmonic mean size over time (Motro and Thomson 1982), which is closer to the low-phase population size than the high-phase. The overall potential for higher effective population size (Table 4)—shorter generation time, higher litter size, and greater per-female reproductive output—may be such that even low-phase effective sizes are bigger than those in

the other study species. For brown lemmings and singing voles, faster mutation rates and increased dispersal during the high-phase of population cycles likely also play a role in maintaining genetic diversity within each clade (Erhich and Jorde 2005). Species-specific diversity changes in response to climate change (Hadly et al. 2004), over both short and long timeframes. Life history traits and effective population size may play a large role in maintaining genetic diversity in the face of climate warming. Understanding these effects may be crucial to predicting the genetic responses to climate change in the future.

4.6 Acknowledgements

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4.7 Figures

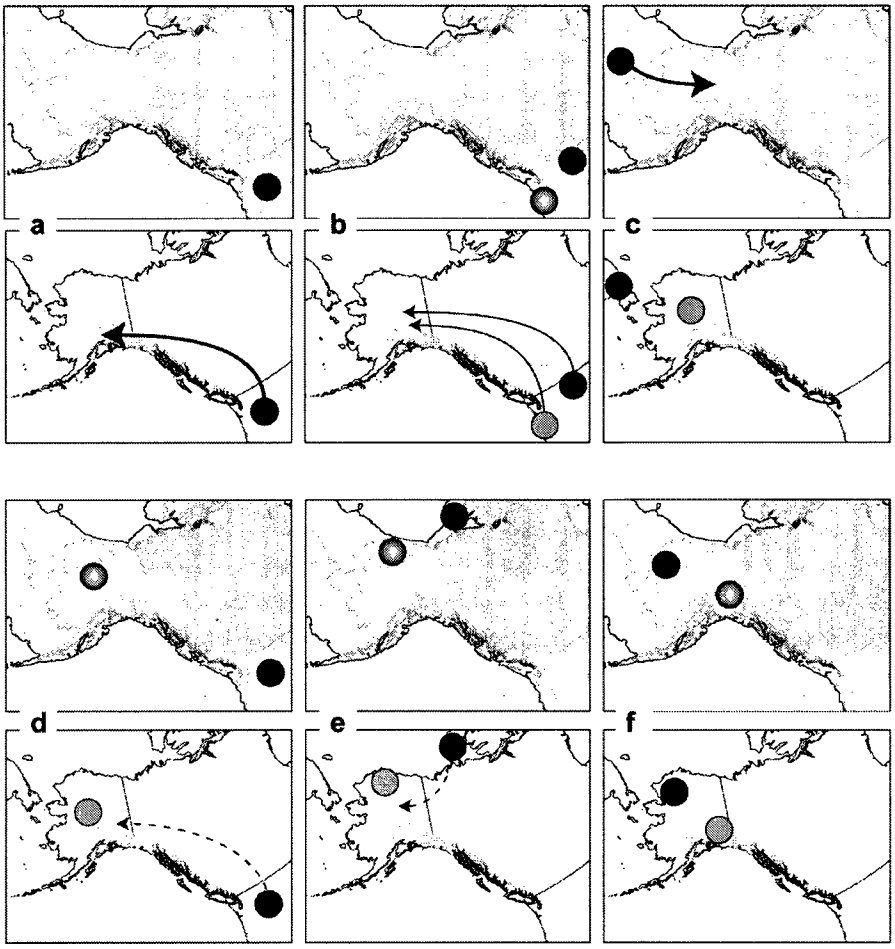

Figure 1 – Beringian phylogeographic patterns

Patterns of ancestral areas and colonization routes (arrows) inferred for mammals of eastern Beringia, adapted from Weksler et al. (accepted). Panels above show glacial extent (grey) at the last glacial maximum and putative population centers (black and variegated). Expected diversification (black and grey; below) under a history of: a) sub-Laurentide colonization via a single route, b) sub-Laurentide colonization via two or more routes, c) recent colonization from Asia, d) vicariance between Beringian and sub-Laurentide populations, e) vicariance between Beringian and high-Arctic populations, and f) intra-Beringian diversification.

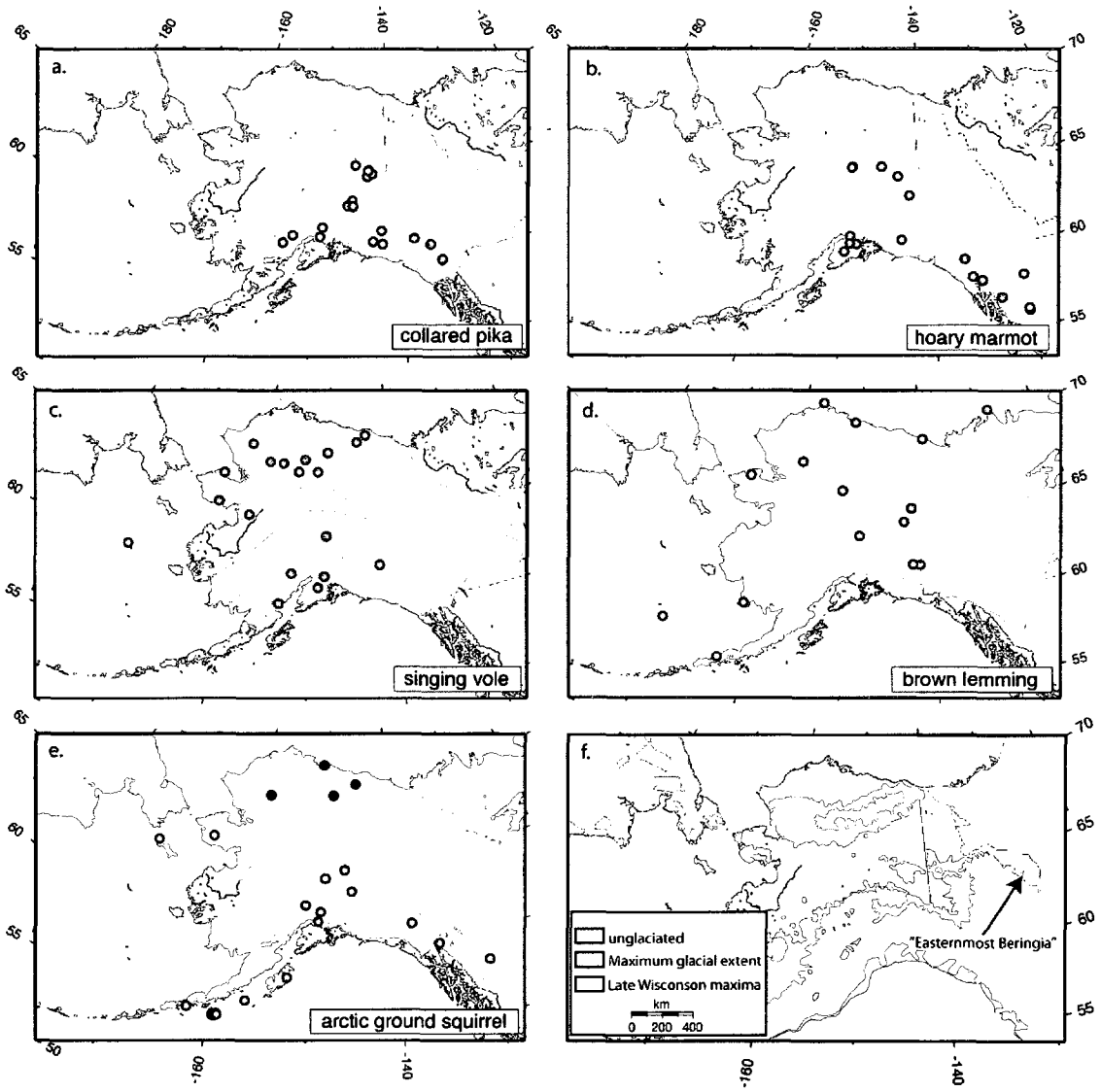

Figure 2 – Species range maps, sampling, and lineage distributions

a.-e.) Range maps (dark grey) and sampling localities showing eastern (red), western (blue), and northern (black, Arctic ground squirrels only) groups. Range maps were modified from NatureServe (http://www.natureserve.org/; accessed 23 May, 2009). f.) Available Beringian habitat during the Pleistocene. Glaciation at the last glacial maximum, the maximum extent of Pleistocene glaciation, and unglaciated areas and the exposed area of the Bering Land Bridge and the ice-free region of Easternmost Beringia are shown (Duk-Rodkin and Hughes 1991; Szeicz and MacDonald 2001; Beierle 2002; Manley and Kaufman 2002).

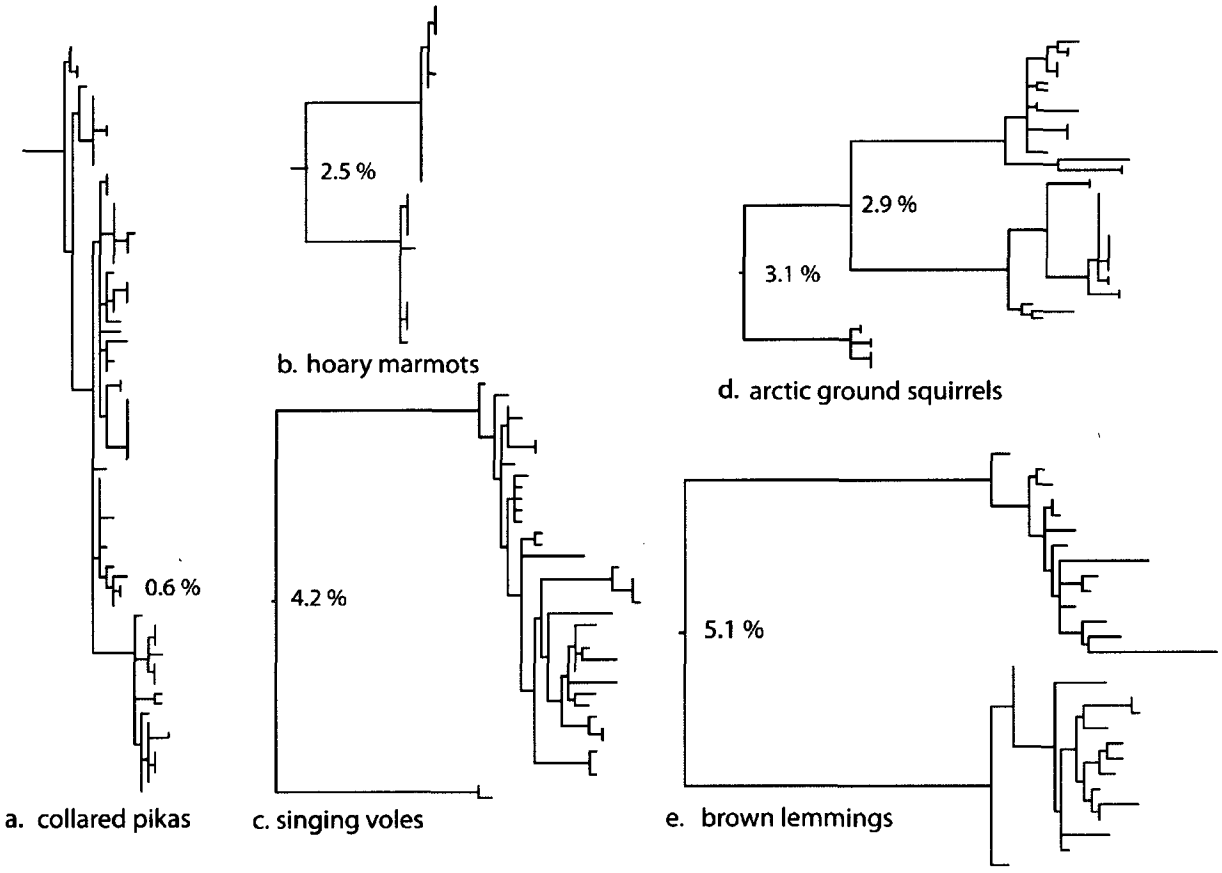

Figure 3 – Scaled likelihood trees

Scaled likelihood trees for a) collared pikas, b) hoary marmots, c) singing voles, d) arctic ground squirrels, and e) brown lemmings. West (above, blue) and east (below, red) lineages are depicted for all species. The northern lineage of arctic ground squirrels is sister to the east-west phylogroups. Pairwise net divergences (*Da* calculated in DnaSP; Librado and Rozas 2009) are shown between clades.

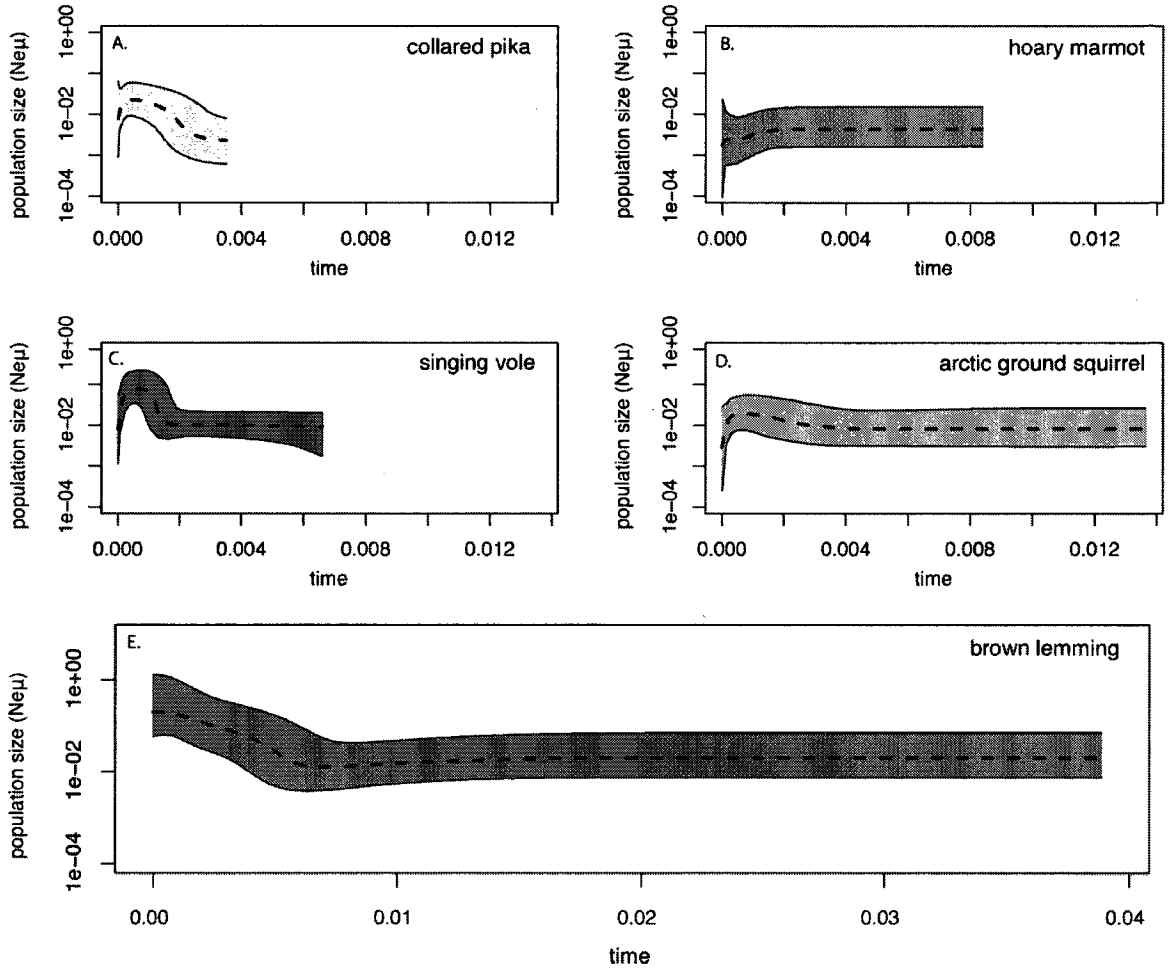

Figure 4 – Bayesian skyline plots

Bayesian skyline plots showing demographic change (in units of effective population size scaled by mutation rate) over time (in substitutions per site) for all five species. Note the difference x- and y- axes for brown lemmings (d), for which a longer population history and a larger scaled effective population size were suggested. The median population size is the center dashed line, the 95% HPD intervals are shown in surrounding grey. Because of the single locus and contemporaneous sampling utilized in this study, only recent demographic trends are evident.

4.8 Tables

Table 1 – Beringian history

Geographic location of sister species, proposed phylogeographic histories, presence of fossils from Beringia, and probability of nunatak survival for the five species included in this study.

	Sister species	**Proposed phylogeographic history**	**Beringian fossils**[12, 13]	**Nunatak survival**
collared pika	North America *O. princeps*[1]	Beringian endemic[6,7]	Early to Middle Pleistocene	yes[14]
hoary marmot	North America *M. vancouverensis*[2]	post-Laurentide colonizer[7]	Late Pleistocene	yes[15]
singing vole	North America *M. xanthognathus*[3]	endemic and colonizer[7,8]	Early to Middle Pleistocene	unknown
brown lemming	Asia *L. sibiricus*[4]	Beringian endemic and colonizer[9,10]	Early to Middle Pleistocene	yes[16]
arctic ground squirrel	North America *S. richardsoni* or *S. elegans*[5]	Beringian endemic[9, 11]	Early to Middle Pleistocene	yes [14]

1. Lanier and Olson 2009
2. Steppan *et al.*, 1999
3. Jaarola et al. 2004
4. Fedorov et al. 2003
5. Herron et al. 2004
6. Guthrie 1973
7. Youngman 1975
8. Weksler et al. accepted
9. Macpherson 1985
10. Fedorov et al. 2003
11. Eddingsaas et al. 2004
12. Kurtén and Anderson 1980
13. MacDonald and Cook 2009
14. Murray and Murray 1969
15. Miller 2006
16. Fedorov and Stenseth 2001

Table 2 – Hypotheses

Expectations of relative genetic diversity, signal of range expansion, and the number of separate divergence times suggested under each historical model (corresponding to patterns f, e, and both e and f)

	Inter-Beringian diversification	Vicariance between Beringia (W) and sub-Laurentide (E_S)	Species-specific patterns W from E or E_S
Expected diversity	$W \cong E$	$W >> E_S$	$W \cong E$, $W >> E_S$
Expected demographic expansion	$W \cong E$	$W << E_S$	$W \cong E$, $W << E_S$
Expected number of simultaneous divergences	1	1	>1

Table 3 – Population genetics diversity metrics

Population genetic summary statistics: n = number of samples, h = number of haplotypes, S = number of segregating sites, θ based on π and a finite sites model, Fu's *Fs*, Fay and Wu's *H*, and Tajima's *D*. Note that there are no values given for Fs, H, or D for the eastern singing vole lineage, because of an insufficient sample size. Test statistics significant at the $p < 0.05$ level (tested with 10,000 coalescent simulations in DnaSP), are shown in bold text.

Species	**Sub-clade**	***n***	***h***	***S***	θ_π	***Fs***	***H***	**Tajima's *D***
collared pika								
	East	19	12	19	0.0042	-3.23	3.187	-0.678
	West	58	27	47	0.0058	**-8.192**	3.6515	-1.231
hoary marmot								
	East	12	5	5	0.0012	-1.079	0.9697	-0.583
	West	14	4	3	0.0009	-0.327	0.2637	0.326
singing vole								
	East	2	2	2	0.0018	-	-	-
	West	35	30	87	0.0121	**-12.499**	**-20.981**	-1.448
brown lemming								
	East	14	14	42	0.0121	**-6.188**	-9.1429	-0.874
	West	13	13	49	0.0122	**-5.429**	-6.987	**-1.555**
arctic ground squirrel								
	East	20	12	39	0.0068	-1.012	0.2421	-1.19
	West	21	8	26	0.0052	1.492	-6.89	-0.702
	North	7	3	5	0.0023	1.853	1	1.28

Table 4 – Ecology

	Body mass[1]	**Habitat**[2]	**Mating system**	**Reproductive output**	**Age at maturity**	**Population structure**
collared pika	114-135 g	alpine talus	facultative monogamy[4]	1 litter per year[9]; 2 to 4[9] young per litter	1 year[9]	relatively stable metapopulation[11]
hoary marmot	3.7-5.6 kg	alpine meadow	monogamy, polygamy[5]	0.5 to 1 litter per year [5]; 2 to 5[5] young per litter	3+ years[5]	metapopulation or source-sink[12]
singing voles	15-35 g	alpine or arctic tundra	promiscuous[6]	> 1 litter per year[10]; 6 to 8[1] young per litter	< 1 year[10]	variable colonies (10x amplitude) [8]
brown lemming	26-42 g	alpine, arctic, & maritime tundra	promiscuous[7]	> 1 litter per year; active in winter[7]; 4 to 6[1] young per litter	< 1 year[7]	50 – 125x highly amplitude cycles[10,13]
arctic ground squirrel	250-621 g	alpine & arctic tundra; boreal forest[3]	promiscuous[8]	1 litter per year[8]; 6 to 8[3] young per litter	1 year[3]	source-sink dynamics[3]

1. From 1st to 3rd quartiles based on University of Alaska Museum specimen records
2. MacDonald and Cook 2009
3. Gillis et al. 2005
4. Smith 2008
5. Barash 1973; Kyle et al. 2007
6. Batzli and Henttonen 1993
7. Stenseth and Ims 1993
8. McLean 1983
9. Franken and Hik 2004b
10. Batzli and Henttonen 1990
11. Franken and Hik 2004a
12. Griffin et al. 2008
13. Erhich and Jorde 2005

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4.9 Appendices

Appendix A - GenBank accession number (for published studies) or specimen ID (University of Alaska Museum or Hik = sample ID from the Hik lab at the University of Alberta Edmonton) for samples used in this study. Samples are sorted by species and haplogroup. Starred (*) individuals excluded from the msBayes analyses but included in summary statistic calculations.

Brown lemming (*Lemmus trimucronatus*) – Fedorov *et al.* (2003)

West n = 14: AJ012675; AY219171; AY219164; AY219169; AY219149; AY219168; AY219158; AY219154; AY219147; AY219153; AF119276; AY219148; AY219146; AY219152

East n = 14: AY219170; AY219167; AY219166; AY219165; AY219163; AY219162; AY219161; AY219160; AY219159; AY219157; AY219156; AY219155; AY219151; AY219150

Hoary Marmot (*Marmota caligata*)

West n = 14: 31724; 85858; 85859; 86413; 30932; 33803; 98299; 65635; 86414; 53836; 58238; 58239; 58240; 58241

East n = 12: AF143920; 32649; 35129; 35130; 38302; 38303; 38304; 49848; 24122; 22914; 57693; 78240

Singing vole (*Microtus miurus*) – Weksler *et al.* (accepted)

West n = 35: 68097*; 68104*; 51869; 53080; 75332*; 75345*; 75427; 76019; 76039*; 78042*; 78051; 78108*; 78041; 56150; 56154*; 56383*; 56354; 85562; 85818*; 64503; 64389*; 64406*; 64353; 64357*; 81971; 64375; 81979; 79610; 79805*; 78877; 78878*; 78692; 98727; 56352; 98356

East n = 2; 57749; 57771

Collared pika (*Ochotona collaris*)

West n = 58; 67030*; 31647*; 58208*; 58546; 58548*; 58550*; 56067; 63938; 58257*; 58552; 58216; 58219*; 102554*; AF69794*; 102486*; 102500*; 102492*; 102505*; 102507*; 102495*; 102506; 102496*; 102488*; 100776; 58204*; 63931; 58445; 63932*; 63936*; 31646; 57694; 58213*; 58245*; 58243; 58251*; 58338*; 58422*; 51297*; 58250*; 58407*; 58448*; 58211; 58242; 58316*; 102497*; 100943; 100839*; 100795; 102478*; 102564; 102566*; 102567; 102429*; 102422*; 102434*; 102435; 63946*; 69347*

East n = 19; 102416; 102419; 102420; 102430; 102431; 102432; 102438; 35126; 51896; 71652; Hik 441; Hik 446; Hik 492; Hik 499; Hik 1355; Hik 1385; Hik 1555; Hik 1628; Hik 1649

Arctic ground squirrel (*Spermophilus parryii*) – Eddingsaas *et al.* (2004)

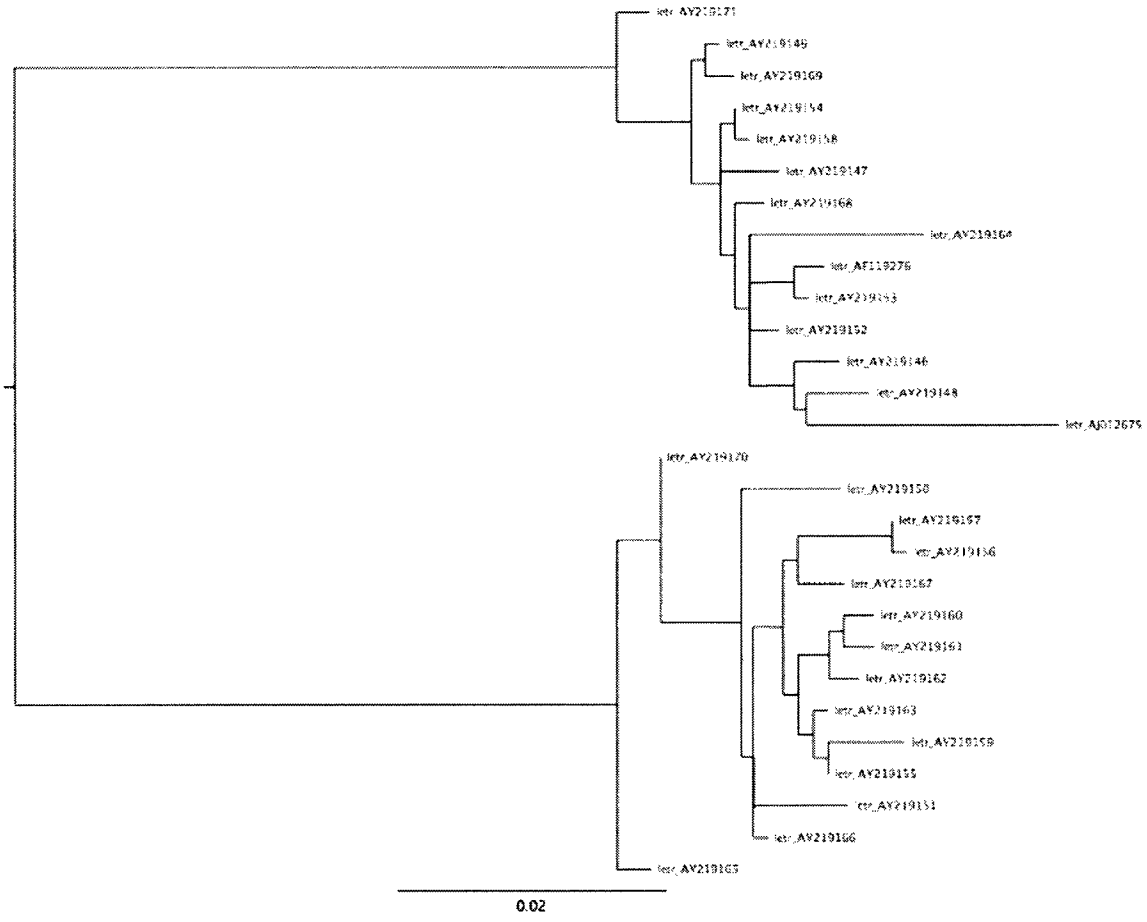
West n = 21: AY427997; AY427996; AY427995; AY427994; AY427993; AY427992; AY427991; AY427990; AY427982; AY427981; AY427979; AY427978; AY427977; AY428007; AY428006; AY428005; AY428004; AY428003; AY428002; AY428001; AY428000

East n = 20: AY428024; AY428023; AY428022; AY428021; AY428020; AY428019; AY428018; AY428017; AY428016; AY428015; AY428014; AY428013; AY428012; AY428011; AY428010; AY428009; AY428008; AY427999; AY427998; AY427980

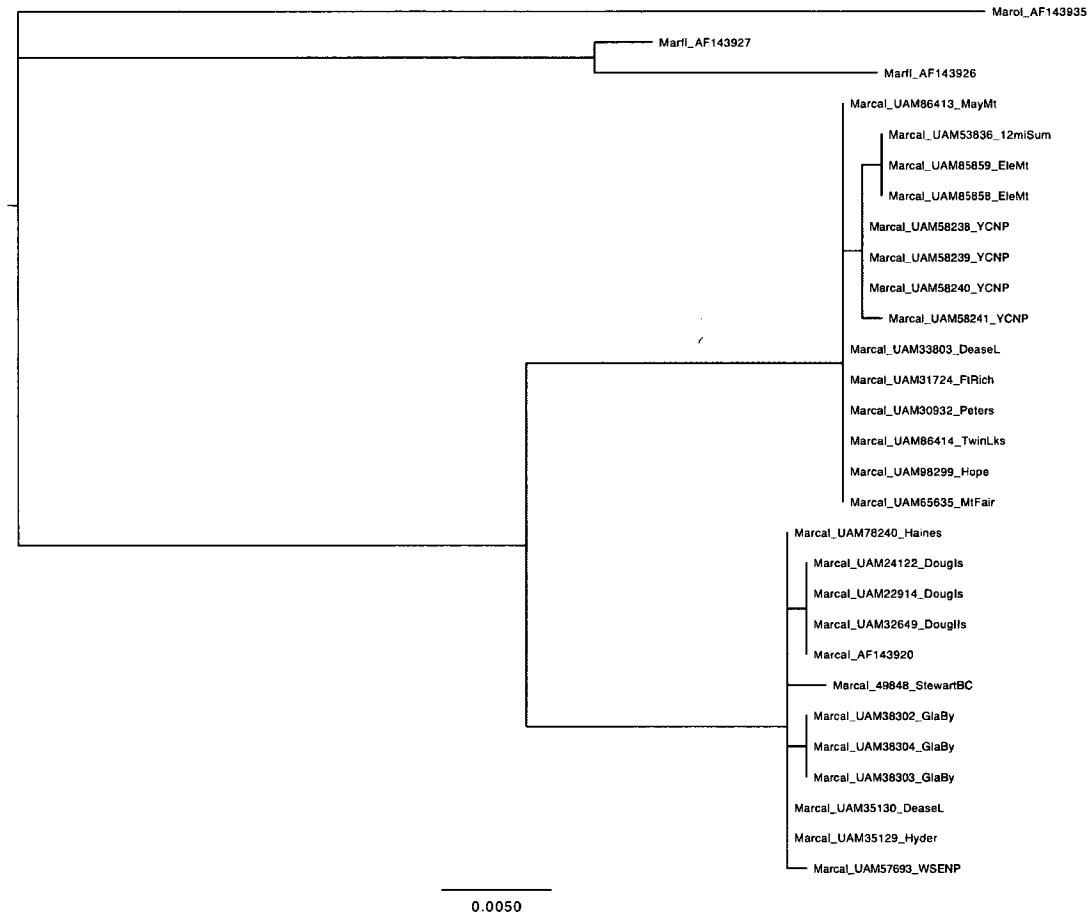
North n = 7: AY427989; AY427988; AY427987; AY427986; AY427985; AY427984; AY427983

Appendix B - Preferred model of evolution (from jModelTest) and maximum likelihood trees

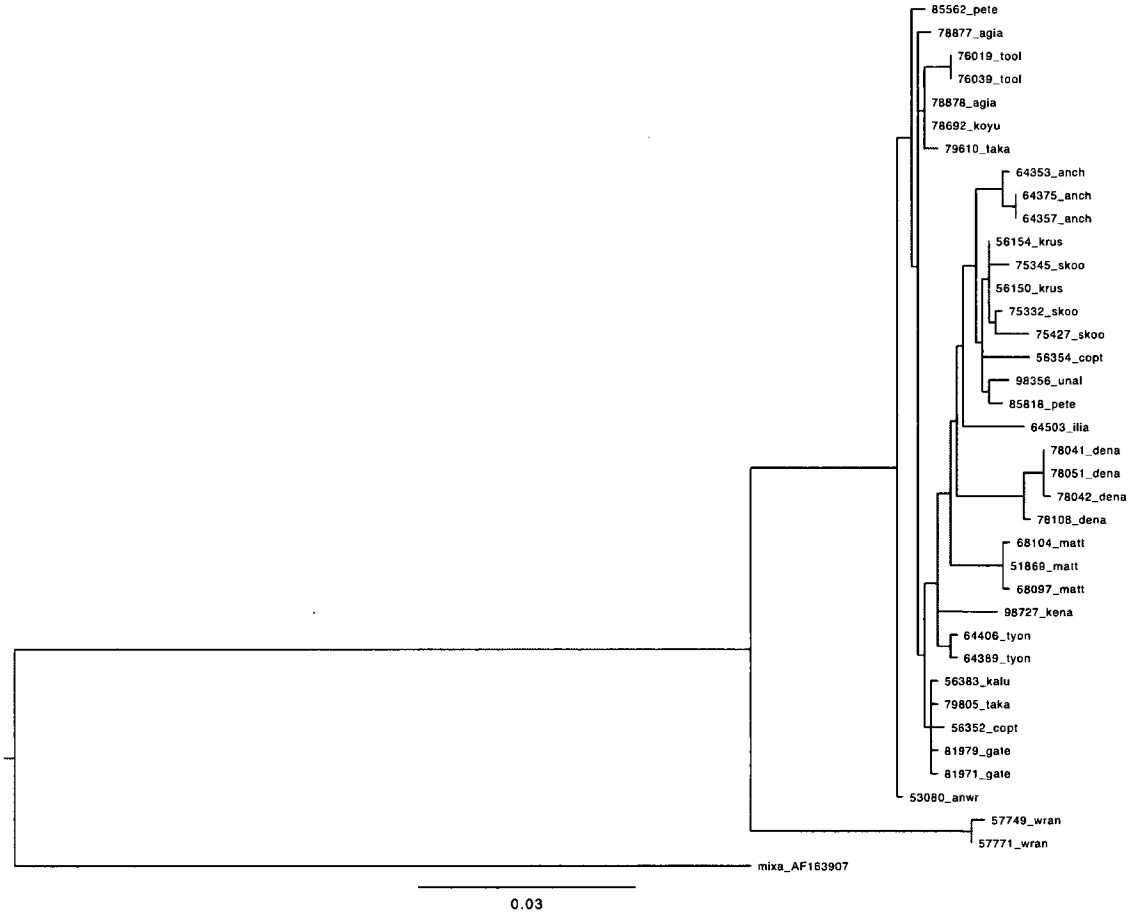
Brown lemming (*Lemmus trimucronatus*) - TIM2+I+G



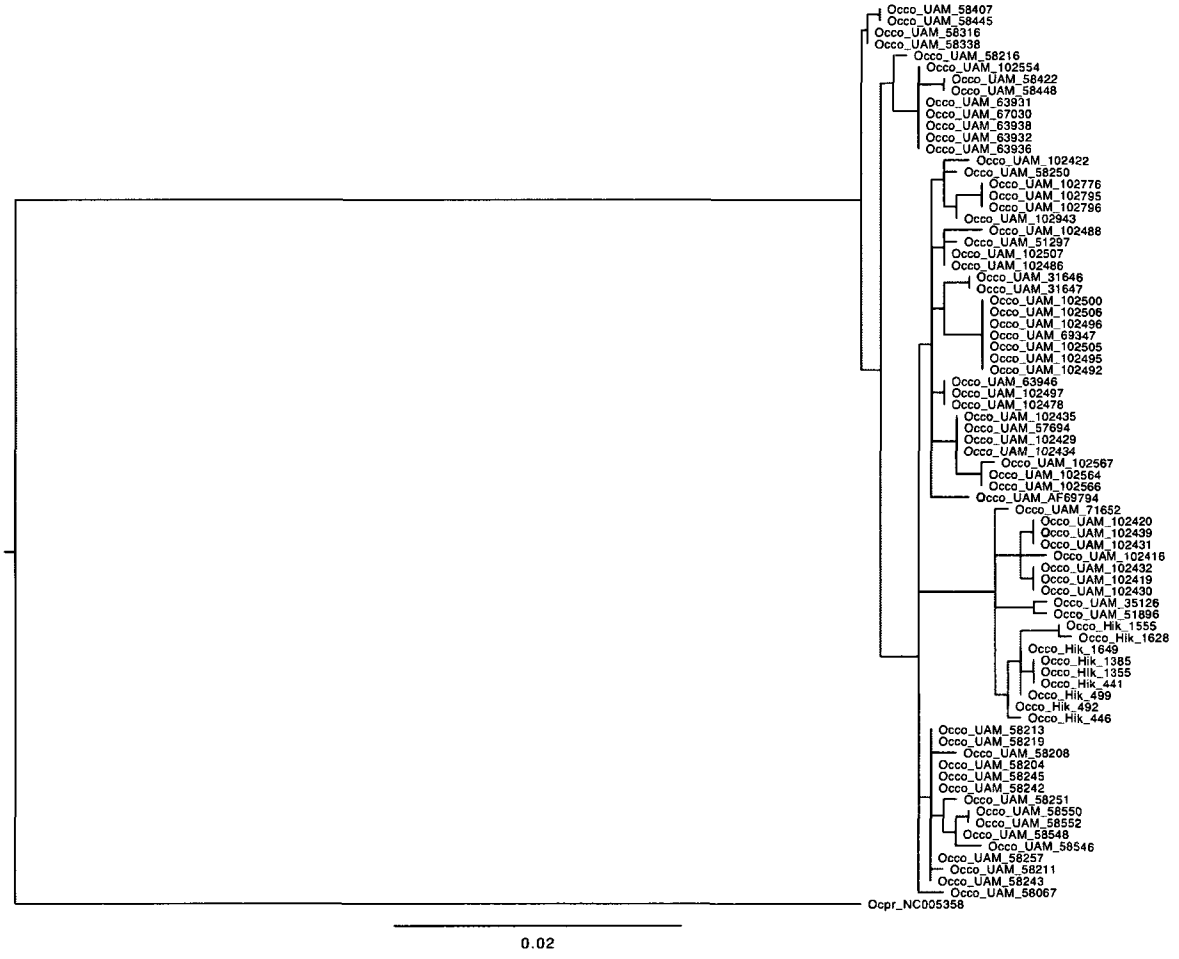
Hoary marmot (*Marmota caligata*) - TrN+G



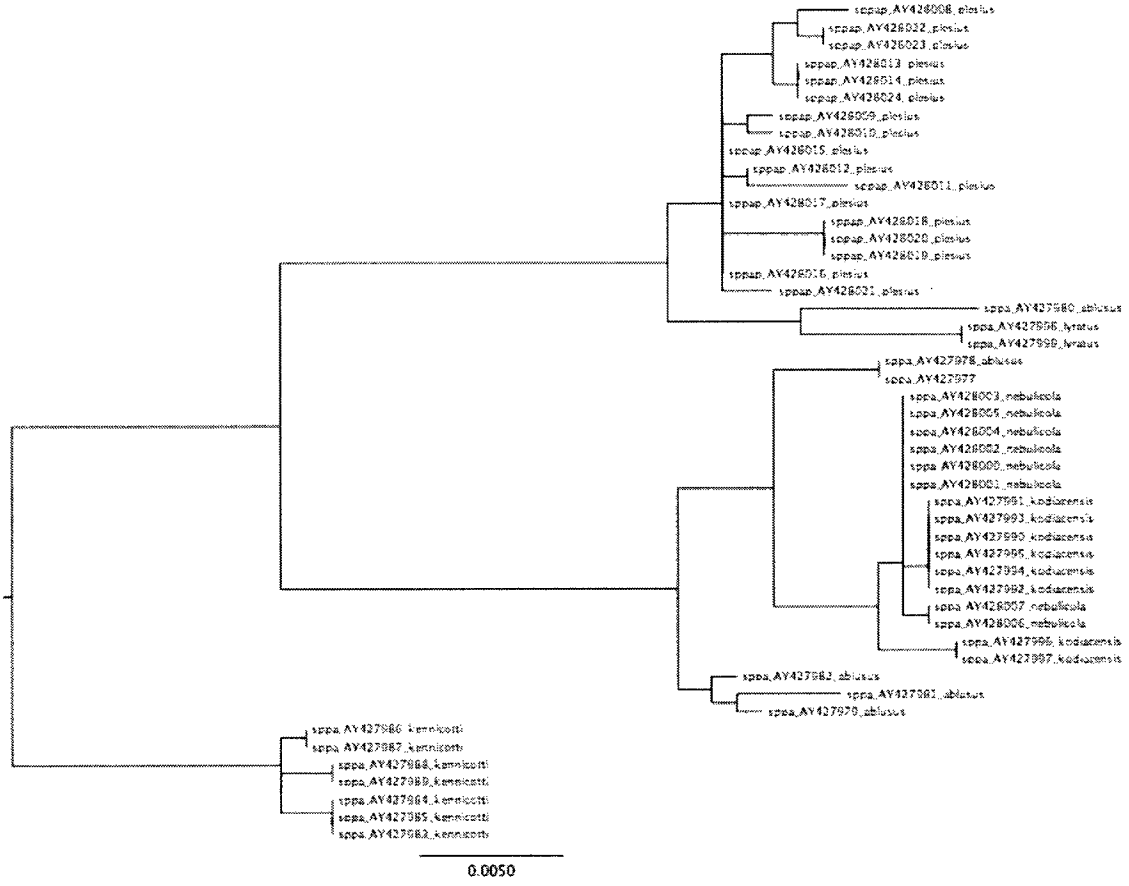
Singing vole (*Microtus miurus*) - TIM1+I



Collared pika (*Ochotona collaris*) - TIM3+I+G



Arctic ground squirrel (*Spermophilus parryii*) - TIM1+I+G



Conclusions:

The low genetic and morphological variation collared pikas (*Ochotona collaris*) exhibit is the result of a low intrinsic variability that has been further eroded by historical demographic processes (such as successive bottlenecks) acting across naturally fragmented habitats over time. In my dissertation research, I have examined the historical and geographic context of processes that structure variation in collared pikas (Chapter 1), changes in collared pika body size (Chapter 2), phylogeographic structuring (Chapter 3), and comparative phylogeography (Chapter 4). By comparing and contextualizing the degree of variation and geographic structure collared pikas exhibit, I hope to distinguish between idiosyncratic patterns and those resulting from phylogenetic or historical factors. Collared pikas and their sister species, the American pika (*O. princeps*), have not shared a common ancestor in at least 2.8 million years (Chapter 1; Lanier & Olson, 2009), but over that timeframe American pikas have accumulated or retained a greater amount of genetic diversity (Chapter 3), call variation (Trefry & Hik, 2009), and parasite fauna (Hobbs, 1980). One potential explanation for the disparity in diversity is the extreme fluctuation in the amount of available habitat associated with Quaternary glacial cycling at higher latitudes (Hewitt, 1996). Habitat change in eastern Beringia has been dramatic over recent (Parmesan & Yohe, 2002; Post *et al.*, 2009) and geologic time frames (Hopkins *et al.*, 1982; Pielou, 1991). Successive demographic contractions occurring at glacial maxima, when ice cover made many areas inaccessible, and interglacial maxima (glacial minima), when pika habitat is maximally fragmented, were more extreme at higher latitudes than at lower latitudes (Pielou, 1991).

In Chapter 1, I established a maximum timeframe for Nearctic pika divergence, and found that the two species last shared a common ancestor prior to the Pleistocene. This refuted previously advanced hypotheses of divergence dating

to the Wisconsin glaciation (Guthrie, 1973). Additionally, I used genetic assessment to examine *O. collaris* and *O. hyperborea*, the northern pika, for evidence of additional trans-Beringian lineages. Although a number of other mammalian species show recent trans-Beringian lineages (Fedorov *et al.*, 2003; Galbreath & Cook, 2004; Waltari & Cook, 2005), pikas show no evidence of trans-Beringian gene flow (Lanier & Olson, 2009). Furthermore, the "universal" mitochondrial molecular clocks applied in other studies of *Ochotona* (Yu *et al.*, 2000; Niu *et al.*, 2004) are too fast, and consistently underestimate divergence dates when compared to fossil estimates. Based on limited geographic sampling, collared pikas exhibit less diversity and more recent coalescences than do American pikas (Chapter 1).

Population structure, connectivity and demography play a role in the accumulation of variation within any species (Allendorf & Luikart, 2007). In Chapter 3, I used mitochondrial DNA from collared pikas throughout their range to examine phylogeographic structure and population connectivity. Based on Φ_{st} and few shared haplotypes, *O. collaris* shows low connectivity between populations. Variation was mostly structured within populations (as opposed to between populations or mountain ranges). Populations of *O. collaris* are less geographically structured and more closely related to one another than are populations of any other pika species studied to date. My analyses revealed divergent two phylogeographical groups: one in the Wrangell-St. Elias and Coast mountain ranges, and the other from the rest of their range (Chapter 3). Phylogeographic subdivision may be the result of localized differentiation in cryptic Pleistocene refugia (Chapter 4).

Several previously studied mammals show similar phylogeographic structure, with a divergent lineage that occurs or contacts a main lineage in Wrangell-St. Elias National Park and Preserve (Fedorov *et al.*, 2003; Weksler *et al.*, accepted). I analyzed my collared pika results (Chapter 3) with four co-occurring mammal species in a comparative phylogeographic context to look for

commonalities in historical demography and geographic structure. While there were species-specific differences in the degree of geographic structure and inferred ancestral localities, all five species show evidence of simultaneous vicariant patterns. Inferred demographic trends in collared pikas, singing voles, and Arctic ground squirrels (Chapter 4) indicate a shared response to ecological changes in their preferred habitat (Guthrie, 1982; Zazula *et al.*, 2007), with historic increases and recent contraction in population size. Future change in alpine habitat is predicted to be extreme, further stressing alpine organisms (Krajick, 2004; Parmesan, 2006). Alpine species, such as *O. collaris*, will need to shift their ranges or adapt to avoid extinction. The degree to which they are able to adapt to climate change is dependent on the intrinsic variation, population connectivity, and capacity for behavioral and phenological buffering they possess.

While genetic variation can provide information about long-term population changes, morphological variation can respond more quickly to climatic and habitat shifts. In Chapter 2, I examined geographic variation in *O. collaris* skull and body size from throughout their range and discovered that they show clinal variation, with size inversely related to latitude. When variation in size is examined by year, pikas are getting slightly larger in size over time, which would be expected if warming essentially resulted in a latitudinal shift in climate regime. However, further examination of climatic factors suggests that temporal variation in body size is better explained by temperature fluctuation. When models including climatic variables were compared, there was evidence of larger body sizes corresponding to high winter temperature anomalies in the Northern Hemisphere and smaller body sizes corresponding to increases in summer temperature in Alaska. In a comparison of pikas from a site sampled in 1962 and 2007, the recent sample had larger skulls and shorter total body lengths than the historic sample.

Low genetic and morphological variation has implications for long-term viability and future management. When compared to co-occurring and con-generic

mammals, collared pikas exhibit low (within-species) divergence and low nucleotide diversity. Based on the number of described subspecies (Hall, 1981; Smith *et al.*, 1990), they also exhibit lower morphological diversity. For alpine mammals of eastern Beringia, there is strong support for simultaneous vicariance structuring major phylogeographic groups (Chapter 4). Over the same length of time, other co-occurring species have acquired or retained greater morphological and/or nucleotide variation than collared pikas. Some pika-specific, or *O. collaris* specific, characteristics may be the cause of this low diversity: lower long-term effective population size, lower mutation rate, and/or population structure. Low genetic variation at loci that control ecologically relevant traits can have negative consequences for adaptation even when neutral loci show variation (Hoffmann *et al.*, 2003). The low variation in mtDNA, a putatively neutral marker, may be indicative of low potential for adaptation if it is related to low genetic variance at ecologically important loci (Kellermann *et al.*, 2009). Strong selective events occurring in collared pikas (Chapter 2; Smith *et al.*, 2004; Morrison & Hik, 2007) will tend to purge genetic diversity, especially when they are the result of temporally fluctuating selection during differing seasons (Futuyma, 1998). Low genetic diversity at ecologically important loci coupled with low connectivity between populations may mean collared pikas will have difficulty adapting to climate change.

Future Directions

Further studies of habitat connectivity and habitat preferences within *O. collaris* will be necessary to better assess the degree to which populations may exchange migrants. We have a general view of habitat preferences within *O. collaris* without a strong understanding of the ecological mechanisms. Several regions appear to be inaccessible to pikas, such as the Kenai Peninsula and central British Columbia. These regions exhibit similar talus, vegetation, and mammalian communities as areas that harbor populations of collared pikas. As they are otherwise connected by apparently suitable habitat to currently occupied regions, there may be as-yet

uncharacterized climatic factors limiting the distribution of *O. collaris*. Understanding the degree and scale of population connectivity is also important for assessing the impacts of climate change on *O. collaris*. Genetic assessments using microsatellites to examine fine-scale connectivity between populations might help us to predict the long-term implications of further habitat fragmentation as shrub- and tree-lines creep skyward (Sturm *et al.*, 2001). Campaigns to list the American pika (*O. princeps*) as threatened by global warming have been unsuccessful, but continued monitoring of both species is still critical.

Future studies are also needed to examine the role of glaciers and nunataks in structuring genetic variation in collared pikas and other alpine organisms. Several species have sutures between phylogroups that occur in the Wrangell-St. Elias region (Chapter 4). A fine-scale study of the distribution of genetic variation in this region relative to current and previous glacial margins would be useful for assessing whether they are barriers or corridors for dispersal. Nunatak populations are also of interest (Chapter 3 & 4), as they might represent interglacial refugia (deeply divergent lineages maintained in isolation) or population sinks that must be constantly replenished from larger sources ("mainland" populations on glacial margins). The continued tracking of population persistence and morphological change through the resurveying of historic populations, will also be important for assessing the importance of midwinter snowmelt and summer warming.

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